Volume LXXIII Number 5

THE BOTANICAL GAZETTE

Editor: JOHN M. COULTER

MAY 1922

Cytology of Chlorophyll Types of Maize - - - - - L. F. Randolph 337
(With Plates XI–XVI)

Cultivation of Excised Root Tips and Stem Tips under Sterile Conditions - - - - - - - - - - William J. Robbins 376
(With four figures)

Influence of Wheat Seedlings upon the Hydrogen Ion Concentration of Nutrient Solutions - - - - Linus H. Jones and John W. Shive 391

Sulphur and Nitrogen Content of Alfalfa Grown under Various Conditions. Contributions from the Hull Botanical Laboratory 294
E. H. Hall 401

Current Literature
Book Reviews - - - - - - - - - - - - - 412
Minor Notices - - - - - - - - - - - - - 415
Notes for Students - - - - - - - - - - - - 416

The University of Chicago Press
CHICAGO, ILLINOIS, U.S.A.

THE CAMBRIDGE UNIVERSITY PRESS, *London*
THE MARUZEN-KABUSHIKI-KAISHA, *Tokyo, Osaka, Kyoto, Fukuoka, Sendai*
THE MISSION BOOK COMPANY, *Shanghai*

MAY 26 1922 LIBRARY

Volume LXXIII Number 5

THE BOTANICAL GAZETTE

A MONTHLY JOURNAL EMBRACING ALL DEPARTMENTS OF BOTANICAL SCIENCE

EDITED BY

JOHN M. COULTER

With the assistance of other members of the botanical staff of the University of Chicago

Issued May 16, 1922

The Botanical Gazette is published monthly by the University of Chicago at the University Press, 5750 Ellis Avenue, Chicago, Ill. ¶The subscription price is $9.00 per year; the price of single copies is 75 cents. Orders for service of less than a half-year will be charged at the single-copy rate. ¶Postage is prepaid by the publishers on all orders from the United States, Mexico, Cuba, Porto Rico, Panama Canal Zone, Republic of Panama, Dominican Republic, El Salvador, Bolivia, Colombia, Honduras, Nicaragua, Peru, Hawaiian Islands, Philippine Islands, Guam, Samoan Islands, Shanghai. ¶Postage is charged extra as follows: For Canada, 30 cents on annual subscriptions (total $9.30); on single copies 3 cents (total 78 cents); for all other countries in the Postal Union, 60 cents on annual subscriptions (total $9.60), on single copies 6 cents (total 81 cents). ¶Patrons are requested to make all remittances payable to The University of Chicago Press in postal or express money orders or bank drafts.

The following are authorized to quote the prices indicated:

For the British Empire: The Cambridge University Press, Fetter Lane, London, E.C. 4, England. Yearly subscriptions, including postage, 57*s.* 7*d.* each; single copies, including postage, 4*s.* 10*d.* each.

For Japan and Korea: The Maruzen-Kabushiki-Kaisha, 11 to 16 Nihonbashi Tori Sanchome, Tokyo, Japan. Yearly subscriptions, including postage, Yen 19.28 each; single copies, including postage, Yen 1.63 each.

For China: The Mission Book Company, 13 North Szechuen Road, Shanghai. Yearly subscriptions, $9.00; single copies, 75 cents, or their equivalents in Chinese money. Postage extra, if mailed direct outside of Shanghai, on yearly subscriptions 60 cents, on single copies 6 cents.

Claims for missing numbers should be made within the month following the regular month of publication. The publishers expect to supply missing numbers free only when losses have been sustained in transit and when the reserve stock will permit.

Business correspondence should be addressed to The University of Chicago Press, Chicago, Ill.

Communications for the editor and manuscripts should be addressed to the Editor of the BOTANICAL GAZETTE, The University of Chicago, Chicago, Ill.

Contributors are requested to write scientific and proper names with particular care, to use the metric system of weights and measures, and in citations to follow the form shown in the pages of the BOTANICAL GAZETTE.

Papers in excess of thirty-two printed pages are not accepted unless the author is willing to pay the cost of the additional pages, in which case the number of the pages in the volume is correspondingly increased. Carbon copies of manuscripts will not be accepted.

Illustrations are furnished without cost to author only when suitable originals are supplied. Plates in excess of four are not accepted unless the author is willing to bear the additional cost. A copy of the suggestions made in the April number 1916, will be sent on application. It is advisable to confer with the editors as to illustrations required in any article to be offered.

Separates if desired, must be ordered in advance of publication. A table showing approximate cost of separates is printed on an order blank which accompanies the proof; a copy will be sent on request.

Entered as second-class matter August 21, 1896, at the Post-Office at Chicago, Illinois, under the Act of March 3, 1879.

Acceptance for mailing at special rate of postage provided for in Section 1103, Act of October 3, 1917, authorized on July 15 1918.

PRINTED IN THE U.S.A.

A Living Monument to

AGASSIZ

A Joy and Benefit to Students and Lovers of Nature

Please co-operate in the great work of

THE AGASSIZ ASSOCIATION, Inc.

Forty-seven years of effective service
Science, Education and Humanity

The Guide to Nature

Monthly Illustrated Magazine

EDWARD F. BIGELOW, Editor

Subscription, $1.50 per year; single copy, 15 cents; three months' trial, 25 cents

Please write for particulars

ARCADIA
SOUND BEACH CONNECTICUT

?

Have You Read

Evolution, Genetics, and Eugenics

By

HORATIO HACKETT NEWMAN

Most thinking people today are more or less interested in evolution and eugenics. This volume by Mr. Newman will be found of unusual significance, as it is written in an easy-reading style and contains excerpts from such well-known writers as Darwin, Wallace, Romanes, De Vries, Le Conte, Weismann, Morgan, Castle, Lull, Guyer, Popenoe, Johnson, Babcock, Clausen, Walter, Saleeby, Shull, Tayler, Downing, and Thomson.

This book has been prepared to meet a specific demand for an account of the various phases of evolutionary biology condensed within the scope of one volume of moderate size.

xviii+524 pages, 8vo, cloth, $3.75, postpaid $3.90

The University of Chicago Press
Chicago - - - - Illinois

Second Impression

The Anatomy of Woody Plants

(*With 306 figures and illustrations*)

By E. C. JEFFREY
Professor of Plant Morphology in Harvard University

The author is easily the leading authority on the anatomy of plants. His book aims to describe, analyze, and discuss the anatomical structure of the most important families of plants. It does for the anatomy of plants what so many textbooks have done for the human anatomy. The book will be of great service, not only to botanical instruction everywhere, but also to paleobotany, for the study of vascular anatomy has revolutionized that subject.

x+478 pages, cloth; $4.75, postpaid $4.95

THE UNIVERSITY OF CHICAGO PRESS
CHICAGO - - - - - - - - - ILLINOIS

MORPHOLOGY *of* GYMNOSPERMS

By JOHN M. COULTER *and* CHARLES J. CHAMBERLAIN

A revised edition of Coulter and Chamberlain's well-known work on *Morphology of Gymnosperms* is now offered, not only for the use of investigators, but for advanced students in this field.

Each of the seven great groups is presented in detail, and the final chapter discusses the problem of phylogeny and points out the evolutionary tendency. Although some corrections and additions occur in the presentation of all the groups, the most numerous changes will be found in the chapter on Cycadales, the group which has received the most attention since the publication of the former edition. The most important addition is to the Bibliography, 150 titles being added to the 484 titles of the former book. This list will put the student in touch with the work of the last seven years. The book is equipped with an index and appendixes.

"The most important general work on the gymnosperms which has ever appeared."—E. C. JEFFREY in *Science*.

478 pages, 462 illustrations, 8vo, cloth; $5.00, postpaid $5.20

The University of Chicago Press
Chicago - - - - - Illinois

Heredity and Eugenics

By

JOHN M. COULTER, WILLIAM E. CASTLE, CHARLES B. DAVENPORT, EDWARD M. EAST, *and* WILLIAM L. TOWER

¶ Leading investigators, representing the University of Chicago, Harvard University, and the Carnegie Institution of Washington, have contributed to this work. Great care has been taken by each contributor to make clear to the general reader the present position of evolution, the results of experiments in heredity in connection with both plants and animals, and the enormous value of the practical application of these laws in breeding and in human eugenics. The volume is profusely illustrated.

viii+316 pages, 8vo, cloth; $3.00, postpaid $3.20

The University of Chicago Press
Chicago - - - - - Illinois

New and Revised Edition of a Standard Work

METHODS IN PLANT HISTOLOGY

By CHARLES JOSEPH CHAMBERLAIN, PH.D.
Professor of Botany in the University of Chicago

This book (third edition, completely revised) contains directions for collecting and preparing plant material for microscopic investigation. Killing and fixing, freehand sectioning, the paraffin method, the Venetian turpentine method, the celloidin method, and the glycerin method are treated in detail, and chapters on making photo-micrographs and lantern slides have been added. In later chapters specific directions are given for making such preparations as are needed in a study of the plant kingdom from the algae up to the flowering plants. Ten years' experience with the Venetian turpentine method has brought it to a high degree of efficiency; the paraffin method has been greatly improved; the notes on staining have kept pace with cytological research, and the directions for finding material are more extensive.

The book may be used in classes under an instructor, but the directions are so explicit that students working alone should soon learn to make first-class preparations.

With the exception of the sections on the glycerin and celloidin methods, this third edition is practically a new book.

Illustrated, xii+314 pages, 8vo, cloth; $3.25, postpaid $3.45

THE UNIVERSITY OF CHICAGO PRESS
CHICAGO - - - - - - - ILLINOIS

Cambridge University Press

Lichens. By ANNIE LORRAIN SMITH, F.L.S., Acting Assistant, Botanical Department, British Museum. With 135 illustrations. Royal 8vo. Price on application. Cambridge Botanical Handbooks.

Fungi. Ascomycetes, Ustilaginales, Uredinales. By HELEN GWYNNE-VAUGHAN, D.B.E., LL.D., D.Sc., F.L.S. With a frontispiece and 196 text-figures. Royal 8vo. Price on application. Cambridge Botanical Handbooks.

The Classification of Flowering Plants. By ALFRED BARTON RENDLE, M.A., D.Sc., F.L.S., Keeper of the Department of Botany, British Museum. Vol. I. Gymnosperms and Monocotyledons. Demy 8vo. With 187 illustrations. $5.00. Cambridge Biological Series.

A Manual and Dictionary of the Flowering Plants and Ferns. By J. C. WILLIS, M.A., Sc.D. Fourth edition. Crown 8vo. $8.40. Cambridge Biological Series.

The Elements of Botany. By SIR FRANCIS DARWIN, Sc.D., M.B., F.R.S., Fellow Christ's College. Second edition. With 94 illustrations. Crown 8vo. $2.60. Cambridge Biological Series.

Practical Physiology of Plants. By SIR FRANCIS DARWIN, Sc.D., F.R.S., and E. HAMILTON ACTON, M.A. Third edition. With 45 illustrations. Crown 8vo. $2.10. Cambridge Biological Series.

Botany. A textbook for Senior students. By D. THODAY, M.A., Lecturer in Physiological Botany and Assistant Director of the Botanical Laboratories in the University of Manchester. Revised and enlarged edition. With 230 text-figures. Large crown 8vo. $2.60.

The Ferns of South Africa containing descriptions and figures of the ferns and fern allies of South Africa. By THOMAS R. SIM. Second edition. With 186 plates. Demy 8vo. $10.00.

Mendel's Principles of Heredity. By W. BATESON, M.A., F.R.S., V.M.H., Director of the John Innes Horticultural Institution. Third impression with additions. With 3 portraits, 6 colored plates, and 38 figures. Royal 8vo. $5.00.

Water Plants. A Study of Aquatic Angiosperms. By AGNES ARBER, D.Sc., F.L.S. Royal 8vo. With a frontispiece and 171 text-figures. $10.00.

Devonian Floras. A Study of the Origin of Cormophyta. By E. A. NEWELL ARBER, D.Sc., F.L.S. With a preface by D. H. SCOTT, F.R.S. Demy 8vo. With a frontispiece and 47 text-figures. $7.00.

Practical Plant Biochemistry. By MURIEL WHELDALE ONSLOW. Royal 8vo. $6.40.

Published by the Cambridge University Press (England)
The Macmillan Co., Agents in the United States
64 and 66 Fifth Avenue, New York City

A NEW BOOK FOR EVERY LOVER OF NATURE

A NATURALIST IN THE GREAT LAKES REGION

By ELLIOT R. DOWNING

Associate Professor of Natural Science in the School of Education of the University of Chicago

THE person who loves the out-of-doors and wants to know more about Nature "in her visible forms" will be interested in this new book by Mr. Downing, who is recognized as one of the leading naturalists of the United States. He knows the region "inch by inch" and presents many interesting facts about the various forms of life which inhabit prairie, forest, and dunes.

¶ The volume traces the geological and physiographic development of some of the more important features of the region, showing how the distribution of plants and animals is dependent upon these features.

Profusely illustrated, flexible binding, xxvi+328 pages
$3.50, postpaid $3.65

Take this book with you on your vacation

THE UNIVERSITY OF CHICAGO PRESS
CHICAGO - - - - ILLINOIS

VOLUME LXXIII NUMBER 5

THE

BOTANICAL GAZETTE

May 1922

CYTOLOGY OF CHLOROPHYLL TYPES OF MAIZE

L. F. RANDOLPH

(WITH PLATES XI–XVI)

Introduction

During recent years there have been discovered a number of instances of chlorophyll inheritance which are characterized by features due to unusual modes of chlorophyll distribution and behavior. These phenomena are obviously of interest both to the geneticist and the cytologist; to the geneticist because some of them almost certainly represent distinct categories of inheritance, and to the cytologist because of the manner in which they involve the origin and behavior of cell organs other than the nucleus, which latter has been generally held to be mainly responsible for the transmission and development of inherited characters. Much attention has been devoted to this subject by geneticists, whereas the cytological aspects of the problem have not as yet received as much attention. It has been felt that a more adequate knowledge of the behavior of the visible constituents of the cell is of prime importance in the attempt to find a solution of the problem. The present study was undertaken for the purpose of determining whether or not there are in the cells of certain chlorophyll types of maize any visible structural differences which can be shown to be responsible for, or correlated with, the known genetic behavior of these plants.

TYPES OF PLASTID INHERITANCE

There have been described numerous cases of chlorophyll variation which involve an unequal and frequently a very irregular distribution of green color in various regions of the plant. The inheritance of such color patterns has not been found always to conform to the behavior usually ascribed to Mendelian characters. A number of these cases will be reviewed briefly, and for convenience those which have been reported in maize and other known cases will be considered separately.

PLANTS OTHER THAN MAIZE.—A large number of more or less distinct color patterns have been shown to behave as simple Mendelian recessives. Albino seedlings, devoid of chlorophyll and which consequently die in the seedling stage, have been reported in *Antirrhinum latifolium* and *Melandrium album* by BAUR (**3**), in *Hordeum distichum* by KIESSLING (**38**), and in *Phaseolus vulgaris* by TJEBBES and KOOIMAN (**55**). Pale green seedlings have been described in *Urtica pilulifera* by CORRENS (**6**), and in *Ipomoea hederacea* by MIYAZAWA (**49**). Yellowish green seedlings have been reported in *Mirabilis jalapa xantha* by CORRENS (**7**), and in *Nicotiana rustica* by ALLARD (**1**). Various types of chlorophyll variegation have also been shown to be inherited as simple Mendelian recessives: in *Aquilegia vulgaris* by BAUR (**3**), in *Pisum arvense* by KAJANUS (**37**), and in *Capsella bursa-pastoris* and *Arabis albida* by CORRENS (**8**).

In other cases similar characters do not seem to be inherited in a Mendelian fashion, but are transmitted from one generation to the next through the female parent alone. Since the male parent does not seem to be definitely concerned in the transmission of the character, such cases have been called "maternal inheritance." The first case of this sort was described by CORRENS (**6**) in *Mirabilis jalapa albomaculata*. Plants of this strain produce branches having green leaves, others having white leaves, and still others with leaves which are partly green and partly white. All types of branches occur on the same plant, and all bear flowers. It was found that flowers from a green branch when self-fertilized produced only green seedlings in the following generation, and bred true thereafter. When crosses were made between flowers of green

and white branches occurring on the same plant, the resulting progeny always resembled the branch which produced the female gamete, regardless of the way in which the cross was made. The results obtained showed clearly that, so far as chlorophyll characters were concerned, the offspring were not affected by the pollen. CORRENS explained these results by assuming that the absence of chlorophyll was due to a cytoplasmic disease, which, although manifesting itself in the plastids, may or may not be limited to these organs. The diseased condition is accordingly transmitted from one generation to the next only through the egg cytoplasm, the male parent not affecting the character of the offspring, since no male cytoplasm is brought into the egg at the time of fertilization.

A situation similar to this was described by BAUR (**3**) in *Antirrhinum majus albomaculata* and *Aquilegia vulgaris*, and also by GREGORY (**23**) in *Primula sinensis*. These workers, however, are inclined to the view that two kinds of plastids, diseased and normal ones, become segregated during somatic mitoses to different cells and consequently to different regions of the plant tissue. This results in the variegated appearance common to plants of these strains. The diseased plastids are described by GREGORY (**23**, *pl. 10, fig. 10*) as being pale yellow and smaller than the normal plastids. In young actively growing leaf tissue both kinds are present in the same cell. Another interpretation should be placed on these figures of GREGORY, as will be discussed later.

A somewhat different case is that reported by BAUR (**2**) in *Pelargonium zonale albomarginata*. In this form plants occur which have green branches and entirely white branches. Flowers borne on either green or white branches when self-fertilized produce offspring in succeeding generations which are like the original branch. When, however, crosses are made between green and white branches, mosaic seedlings (green and white) result, regardless of the way in which the cross is made. This case differs from that of *Mirabilis* in that the inheritance is not the maternal type. Here both the male and female gametes must be concerned in the transmission of the character. BAUR is led to assume that plastids rather than the nucleus are directly responsible for this unusual type of inheritance, and that they are brought in by the male

gamete to the cytoplasm of the egg at the time of fertilization. This assumption is directly contradictory to that of CORRENS. BAUR further assumes that there are present in these plants two kinds of plastids, green and colorless ones, which are permanent cell organs and are sorted out and unequally distributed to daughter cells during somatic mitoses. Thus there results a segregation of green and colorless plastids in different parts of the plant, and this is held to account for the absence of chlorophyll in certain regions of the plant. BAUR (4) later reported similar cases in a strain of *Antirrhinum majus albomaculatum* and in *Aquilegia vulgaris*.

Additional evidence has been furnished by IKENO (36), who has worked with variegated races of *Capsicum annuum* and obtained results similar to those of BAUR in *Pelargonium*. Such strains of *Capsicum*, however, differ from *Pelargonium zonale albomarginata* in that all the plants produced show some degree of variegation, although green branches may occur. Furthermore, when pollen is taken from flowers on either variegated or green branches of a variegated strain and used to pollinate flowers on normal green plants, the resulting progeny are always variegated, although to a less degree than in the variegated parents. IKENO believes that this character is not controlled by the nucleus, but by plastids (diseased or normal) which are transmitted from one generation to the next by both parents. An apparently significant fact in this connection is that the two types of hereditary transmission, maternal and biparental, have not been found to occur in the same species. It is possible that this may mean that male cytoplasm regularly enters the egg in some species and not in others; but adequate cytological evidence for this is lacking.

MAIZE.—Numerous cases of chlorophyll inheritance have been reported in maize. Many distinct types have been described which differ greatly, not only in the mode of their inheritance, but also in the amount of chlorophyll present and the distribution and appearance of the pigment during the growth period. EMERSON (20) described several chlorophyll types, and presented evidence to show that albino seedlings conform to a Mendelian type of inheritance, and that the factor concerned is a simple Mendelian recessive. GERNERT (22) presented similar evidence. MILES (47)

furnished additional data on the inheritance of certain types of albinism, and as a result of a cytological investigation of the albino seedlings concluded that plastids are entirely absent in plants of that type. LINDSTROM (43), in a comprehensive study of chlorophyll inheritance in maize, described the behavior of eight distinct types, including those already reported on. In every case the inheritance was shown to be Mendelian. Two of these types, albino and yellow, are seedling characters, the plants failing to mature because of the absence of a sufficient amount of chlorophyll. In a third type, known as "virescent," the seedlings at first are yellowish white, but later become green, and in the mature condition cannot be distinguished from normal green plants. The five other characters described by LINDSTROM as "golden," "greenish-striped," "japonica white striped," "japonica yellow striped," and "fine striped," are manifested only in the mature plants; the young seedlings have a wholly normal appearance. From these studies LINDSTROM concluded that plastid inheritance in maize is typically Mendelian.

At the present time, however, cases are known in which the inheritance of certain aberrant chlorophyll types is not Mendelian. ANDERSON, in genetic studies as yet unpublished, has found that in a certain strain of maize there are produced some plants which are uniformly green, others uniformly yellowish green without sufficient chlorophyll to reach maturity, and still others with distinct yellowish green and green stripes.[1] Breeding experiments so far carried out have failed to show any inheritance of the unusual character through the male parent. The plants which are yellowish green lack a sufficient amount of chlorophyll to reach maturity, and no offspring have been obtained from them. The striped plants, either when self-fertilized or when pollen is used from a green plant of an unaffected strain, produce some green plants, some yellowish green plants, and some striped plants in varying proportions, which seemingly depend upon the amount of yellowish green tissue in the region of the plant producing the ear. Ears have been obtained which have produced only yellowish green

[1] Described in a paper read by E. G. ANDERSON before the Society of American Naturalists at Chicago, December, 1920. A published account is to appear shortly.

plants. The evidence indicates that this is a case of maternal inheritance. In no case has pollen from the affected plants of this strain produced any visible effect when used in crosses on green plants, either in the first or succeeding generations.

It is clearly evident from these cases that the inheritance of chlorophyll variations can hardly be accounted for on the basis of a single explanation. There are widely different categories which at present seem to be entirely distinct. In the case of the Mendelian behavior, the explanations offered are the same as for any other character behaving in a similar manner, although other cytoplasmic organs having a certain degree of individuality are apparently more directly involved in bringing the characters to expression. In this category characters expressed by intracellular organs are apparently under the control of nuclear factors, just as are other Mendelian characters expressed by tissues and multicellular organs. The hypotheses advanced to account for the non-Mendelian behavior of similar characters assume that the plastids themselves are permanent cell organs capable of transmitting certain characteristics, and are not controlled by nuclear factors; and furthermore, that in certain cases plastids are transmitted to succeeding generations by both parents, and in other cases only by the female parent. It must be admitted that such explanations are highly speculative, in view of the number of cytological observations which have so far been made.

METHODS AND TECHNIQUE

The general procedure followed has been to examine the material in the living condition, the observations thus made being supplemented by a study of fixed and stained preparations. It was found that meristematic tissue, including apical meristems and young leaf tips of germinating seeds, could readily be studied in this way. It was necessary in the case of older tissues to remove first the epidermal layer of cells before examining the mesophyll cells beneath. A solution of cane sugar of 7.5–10 per cent concentration was found to be a favorable medium in which to examine fresh material. Cells which are protected by one or two cell layers or by epidermal cells will remain living for a considerable period of

time, even in a water mount. Thus it was found that bits of embryonic (meristematic) tissue will remain living and apparently in an entirely normal condition for 24–36 hours in an isotonic solution of cane sugar. In most cases, however, observations were made immediately after making the mounts in order to avoid the possibilty of any modification of the cell contents. Intra-vitam stains, such as neutral red, Cresyl blue, Janus green B, etc., have been used, but unexpectedly proved to be of relatively little value. Various methods of fixation and staining were used, including those of BENDA, REGAUD, CHAMPY, LAGUESSE, BENSLEY, and other special methods which have been reported as being useful in such studies.

Description

The chlorophyll types here described do not include all the known cases of chlorophyll abnormality in maize, but have been selected as representative of certain distinct categories, illustrating markedly different kinds of behavior, both in development and in inheritance. These types are discussed in genetical literature as "normal green," "Mendelian white," and "Mendelian virescent." The "maternal inheritance strain," discovered by ANDERSON, has not yet been described in the literature.

The development of plastids may readily be followed in the meristematic and mesophyll tissues of young rapidly growing seedlings. In the growth stages immediately following the germination of the seeds, leaf tissue is being formed through the activity of the apical stem meristem, and the embryonic leaves thus formed are increasing rapidly in size through an active division of the cells making up the leaf meristem and through a division and growth of the cells throughout the leaf tissue. The meristematic tissues during these and later stages furnish very favorable material for a study of the cytoplasmic inclusions of the living cells. Since it has been found that the cytoplasmic inclusions of the epidermal cells, as well as those of the vascular system, differ markedly from those of the mesophyll cells in mature leaf tissue, the study of plastid development has been limited to the regions from which mesophyll cells are derived.

Normal green strain

Detailed attention will be given to the development of plastids in a normal green plant, and in the consideration of the other types emphasis will be placed on the points in which such types differ from the normal plants.

Appearance in embryonic cells.—The youngest stages examined were those found in the cells of the promeristematic region of seedlings 36–48 hours after germination. This region occupies the apex of the stem, and is composed of undifferentiated parenchymatous cells which are actively undergoing division in all planes. In undifferentiated tissue in all cases the observations were made on subepidermal cells. In such cells the cytoplasm contains minute granules which may clearly be seen without the aid of special staining methods (fig. 1). These granules appear in the living cell as refringent globules, which are constantly changing their position in the cell as a result of cytoplasmic streaming; this movement seems to be a constant and characteristic feature of actively dividing embryonic cells. The size of these bodies is variable. In the promeristematic region they are rarely more than 1 μ in diameter, and careful observation reveals a closely intergrading series between these and other smaller ones lying just within the lower limit of visibility. Whatever their size, these bodies always appear sharply distinct from the homogeneous cytoplasm in which they are imbedded, and in this material cannot be interpreted as granular constituents of the cytoplasm itself. At this early stage the cytoplasm appears to be entirely free from other accumulations which might be confused with these small bodies.

Appearances which might be interpreted as division stages are frequently seen, both in the living cells and in fixed and stained preparations. In the living cells a condition which strongly suggests division by a simple constriction and bipartition of these bodies is frequently noted, but their extremely minute size and their constant movement make it impossible to follow a single granule for a sufficient length of time to obtain direct evidence for its division. Moreover, chance association and separation are of frequent occurrence, especially in cells displaying active cyto-

plasmic streaming. Association may be but momentary, or granules which have been seen to come together may remain attached for a considerable period of time. Thus when a separation is seen to occur, it is not possible to tell whether one is observing an actual division of a single granule, or a reseparation of two granules formerly distinct. The fact that a paired arrangement occurs much more frequently than do groups of three or groups of four might be considered as suggestive, but in view of the observed behavior described, this can scarcely be considered as convincing proof of division. This point is of interest in view of the undoubted division of similar bodies which have become larger in older cells. The marked variation in the size of these bodies and the fact that evidence for their division in these early stages is uncertain raise the question of their nature, a question which will be taken up in the discussion.

Observations on the living cells afford no evidence for the existence of more than one kind of body in the class of cell elements under discussion. An appearance suggesting the presence of two kinds of bodies, lighter and darker ones, is seen when the cells are examined with lenses of high magnification. This, however, is due to their relative position in the cytoplasm. In the living cells these minute bodies are continually in motion, and as they pass into the plane of focus first appear faintly colored, but when sharply in focus they are much darker in color, then become lighter again as they pass out of focus. Thus a single granule may appear light or dark depending on its position in the cytoplasm. The use of intra-vitam stains, such as are commonly employed in studies of cytoplasmic organization, and the fixing and staining methods which have been shown by recent workers to preserve the cytoplasm faithfully with its various inclusions, have also failed to differentiate distinct kinds. Treatment with osmic acid does not appreciably alter the appearance of these bodies (fig. 52). When treated with BENDA'S fixation and stained with haematoxylin, the cytoplasmic inclusions are well preserved, and their appearance is similar to that of living cells (fig. 53). Since these bodies have been found to occur as a constant feature of the cytoplasm of meristematic cells in maize, and inasmuch as they have been found to be definitely

concerned with the formation of chloroplasts, the term "proplastid" will be used for such bodies. Other cytoplasmic granules and transitory accumulations not concerned with the development of plastids may be present later. Oil globules which can be distinguished from the proplastids by their characteristic highly refractive appearance and microchemical reaction, and other metaplasmic masses in the vacuole (fig. 5) are to be found at certain stages. The chemical nature and significance of these bodies are not known, and a detailed consideration of this phase of the problem is hardly within the scope of the present work.

The position and behavior of the proplastids during cell division have been observed in the living cells. There is apparent no definite sorting out of equal numbers to daughter cells, or more active division of individuals during this period. The proplastids are grouped at opposite ends of the cell during mitosis, and their passive distribution to the daughter cells seems to depend wholly upon their chance positions in the cytoplasm at the time of cell-plate formation.

PLASTID DEVELOPMENT.—The formation of mature functional plastids from minute granular proplastids may readily be followed in the subepidermal cells of the tips of leaf buds forming from the apical meristem. The earlier transitional stages are present in the meristematic cells near the apices of successively older seedling leaves which are growing rapidly, and which are not yet exposed to sunlight. Later stages are found in the leaves which are about to emerge from the surrounding sheath. In passing from the tip of such a leaf toward a point somewhat below the tip a series of stages may be observed. Numerous well developed plastids are present in the mesophyll cells of fully exposed seedling leaves.

In leaf tissue recently formed from the apical meristem the cells contain some proplastids which have increased noticeably in size, and others similar in size and appearance to those found in younger cells (fig. 2). Vacuoles are present in these cells, and the cytoplasm becomes more or less limited to the region surrounding the nucleus and to the periphery of the cell, with connecting strands between. Cytoplasmic streaming, which is commonly active at these stages, carries the proplastids about through the cell, even the largest ones being translocated in this manner.

In somewhat older stages (figs. 3, 4) the largest bodies remain stationary or nearly so, and are not affected by the cytoplasmic streaming. A grouping of proplastids of various sizes is often seen in certain parts of the cell. This most frequently occurs in the parietal portion of the cytoplasm, whereas in certain other cells there may be a grouping of almost all of the proplastids about the nucleus (fig. 51). In the present study these and other modes of grouping have not been correlated with any other phenomenon of cell activity.

Evidence for the division of the developing plastids is not difficult to obtain in the later stages (fig. 5). Cytoplasmic streaming is not so active in such cells, and the proplastids which have reached a size of 2 μ or over may frequently be seen in the process of division.

A variation in the size of the proplastids which are present in a single cell is markedly characteristic of the tissues studied. In such a cell as that of fig. 6 there is a close intergrading series, from proplastids which are so small as to be barely visible, up to others which have a diameter of 4.5–5 μ. The largest proplastids of this cell appear faintly green. The presence of these transitional stages within a single cell furnishes convincing proof of the origin of chloroplasts from bodies which at first are scarcely visible, and which one is led to believe may even arise *de novo* from the cytoplasm. In no case has the presence of green color been found to be associated with the proplastids until they have reached approximately one-half of the size and shape of mature functional plastids. The appearance of starch in the developing proplastids, which sometimes occurs, is perhaps associated in some way with chlorophyll formation.

The final stages in the maturing of chloroplasts may be observed in the cells near the tip of a seedling leaf which is about to emerge from the enveloping sheath (figs. 7, 8). Subepidermal cells near the apex contain a small number of partially developed plastids in which chlorophyll is present, and many smaller proplastids of varying size (fig. 7). Farther back from the tip the mesophyll cells contain an increasingly large number of nearly mature plastids, the majority of which are markedly green, and a diminishing number of proplastids (fig. 8). Finally, in the cells of

mesophyll tissue of well developed seedling leaves which are exposed to sunlight the enlarging plastids have attained a diameter of 7–8 μ. In such cells the plastids are closely packed in the parietal layer of cytoplasm, and are intensely green (fig. 9). In these cells, in addition to the larger plastids there are present a certain number of smaller bodies of varying size which cannot be distinguished from the proplastids found in the earlier stages. This fact seems to indicate that such structures represent partially developed plastids and not bodies of a separate category, as some observers have believed. Thus not only meristematic cells, but cells of mature mesophyll tissue as well, contain proplastids in various stages of enlargement, as well as plastids which have attained the maximum size and color.

The condition found in the mesophyll cells of the leaf tissue of a mature plant is very similar to that just described for fully green seedling leaves. In the former the plastids are slightly larger in the exposed portion of the leaf blade, and the number of proplastids is somewhat smaller than in the latter. In portions of mature leaves, however, which are protected from sunlight by other enveloping leaves or leaf sheaths, the development of the proplastids has not progressed as far as in the exposed portion of the leaf, regardless of the fact that the tissue in question is structurally mature. Chlorophyll is almost or entirely absent in such cells. In passing outward toward the green portion of the leaf, the proplastids become progressively larger, and there is a relatively smaller number of more minute primordia. Chlorophyll makes its appearance with the development of the plastids. Figs. 42–46 illustrate the transitional stages found in such a leaf, and the amount of shading used in representing the proplastids indicates the relative intensity of green color to be found in these stages.

There appears to be a close correlation between the development of plastids and the appearance of chlorophyll in the leaf tissue of normal green plants, whether in seedling stages or in the mature plant tissue. Maturing plastids 4–5 μ in diameter are very faintly green, and as they increase in size the intensity of the green color also increases, until the plastids have attained their maximum dimensions, when they are bright green.

EPIDERMAL CELL INCLUSIONS.—In maize the epidermal cells of leaves rarely contain plastids; if present at all they are few in number. Certain very characteristic structures are present, however, which will be discussed only briefly, inasmuch as they seem to have no direct bearing on the problem of chlorophyll development.

The epidermal cells of the meristematic region may be studied without removing them from the remainder of the leaf tissue. Their walls are not heavily cutinized during their early development, and the cells beneath are sufficiently transparent to permit a careful examination of cytoplasmic structures. The cytoplasm is limited to the parietal region of the cell, except for strands which pass through the large central vacuole to the region occupied by the nucleus near the inner wall. The cytoplasm of cells near the tip of embryonic leaves contains minute granules and short rods (fig. 47). These bodies are very numerous, and are carried rapidly about by the flowing cytoplasm, both rods and granules continually changing their positions. In older cells, that is, those farther removed from the tip, the rods are longer and more numerous (fig. 48). In still older cells many elongate filaments are to be found, as well as numerous granules and short rods (fig. 49). Cells have been observed in which even the elongate filaments are actively translocated by the cytoplasm; a single one may thus be observed for some time. This phenomenon furnishes definite proof that the filaments are structurally distinct elements, and not merely lines of flow, or other artifacts due to fixation. The fact that the filaments are longer and more numerous in the older cells than in meristematic cells suggests that they have resulted from an elongation of shorter ones, and that these in turn may have originated from the granular bodies. Convincing proof of their division has not been obtained. A small number of colorless bodies which resemble the partly developed plastids of mesophyll cells are also found in these cells, but mature green plastids are rarely present. In addition to these structures, oil globules are present which may be distinguished by their greater refringence in living cells and by their characteristic reaction to osmic acid. The relation of these structures found in epidermal cells to the proplastids of mesophyll cells will be discussed later.

MENDELIAN WHITE STRAIN

In the "Mendelian white" strain studied there are completely green and completely white plants. Albinism is here inherited as a simple Mendelian recessive. The green plants are entirely like green plants of normal strains, so far as any visible structures are concerned. Microscopic examination of the cells reveals a series of stages in plastid development which is indistinguishable from the series observed in normal green plants already described. In the white (albino) seedlings proplastids are present in the early embryonic stages just as in the green plants (figs. 10–12). In older tissues (figs. 13–16), however, corresponding to similar regions in a green plant (figs. 7–9), there is a striking difference in the behavior of the proplastids. They increase in size very slowly and irregularly, and although the leaf tissue itself continues to grow and differentiate, doubtless at the expense of reserve food stored in the seed, the proplastids do not develop as rapidly, or in as large numbers, as in the tissues in the same stages of differentiation in a green plant. Granules measuring 0.8–1.2 μ in diameter are found just as in normal green tissue, but development beyond this stage occurs only in a few scattered instances. The few proplastids which do become larger frequently present an abnormal appearance (figs. 14–16). In the living cells they are colorless or nearly so, and may sometimes have a darker irregular mass near the periphery (fig. 15). When fixed according to BENDA'S method and stained with haematoxylin, they bear a striking resemblance to small nuclei, and the irregular mass within these bodies bears a striking resemblance to densely staining nucleoli. When the tissues have reached a stage comparable with that at which chlorophyll appears in a normal green plant, there are very few plastids as large as those in which chlorophyll first develops in the green plant, the cell being characterized rather by a large number of proplastids, a condition suggesting retarded development (fig. 16). There also occur irregularly shaped masses, giving the appearance of degenerating plastids which had become partly mature. Figs. 14 and 15 represent the appearance of the cytoplasmic bodies in mesophyll cells occurring between the vascular bundles of the leaf. Fig. 16 shows the condition which is found in a cell nearer the bundle.

Here the proplastids in general are larger and more numerous, and often faintly yellowish green. This appears to indicate that the first steps of chlorophyll elaboration are initiated in these cells.

Although not any of the plastids are normally green in most of the tissue of the leaf blade, there has been found at the extreme tip of the albino seedling leaf a limited region in which the cells contain green chloroplasts (figs. 17–19). In such a region, even within a single cell, are found transitional stages between colorless partially mature plastids and those which are fully green (fig. 18). Chlorophyll, therefore, is not entirely absent from the albino seedlings of the "Mendelian white" strain. In these albino seedlings, however, there is never developed a sufficient amount of chlorophyll to enable the plant to live beyond the seedling stage. Microscopic examination shows clearly that the initial structural basis for plastid development is present, chloroplasts which are normal in appearance being actually found in some cases. This latter condition never becomes general throughout the plant. It therefore seems clear that in this case failure of the plant to become green is not to be explained as the result of an absence of plastids or plastid primordia.

Mendelian virescent

A third category is "Mendelian virescent." In this strain the affected plants in the young seedling stage resemble those of the "Mendelian white" strain. The seedling leaf is at first white, but later nearly the entire leaf becomes green, the color deepening rapidly. Later formed seedling leaves are somewhat greener in the early stages, while leaves formed toward maturity are entirely green from the start.

In the virescent plants the early stages in the development of the plastids are similar in all respects to the corresponding stages in a normal green plant. So far as it is possible to tell, the number, size, and development of the proplastids is identical in the two cases (figs. 20–23). In the tips of the young leaves, as they increase in size and become exposed to sunlight, the growth of the proplastids and the development of chlorophyll proceed as in green plants (figs. 24–26, 32). In the main portion of the leaf blade, on the

other hand, the mesophyll tissue fails to become green as rapidly as the tissue near the tip, and the cells between the bundles are found to contain only partly developed proplastids (fig. 27). Cells nearer the vascular bundles contain larger plastids with more chlorophyll (fig. 28), while in cells adjoining the bundles the plastids have reached nearly the maximum size and color (fig. 29). In somewhat older leaf tissue which is gradually becoming green there is a corresponding increase in the size of the proplastids (figs. 30–32), and the resulting plastids eventually become fully green (fig. 32). Here, then, is a case in which the usual formation of chloroplasts with their pigment seems to be merely delayed, but, unlike the "Mendelian white" seedlings, development continues until the whole plant becomes fully green.

MATERNAL INHERITANCE STRAIN

In this strain there are plants of three kinds. Some are entirely green and others are uniformly yellowish green; a third class is made up of plants which show longitudinal stripes of the two colors. The number and character of such stripes are extremely variable. Some plants are distinguishable from normal green plants only by the presence of one or two stripes, which may be of any width, and which may be of a color only slightly lighter than normal green. On the other hand, the appearance of some plants is rendered most striking by a large number of stripes, which, although their yellowish green color varies in depth to a certain degree, are never white. Frequently a plant may be divided sharply and almost equally throughout its entire length into green and yellowish green halves. Again, plants are observed which are completely yellowish green, except for one or two fully green stripes. The light stripes, whatever the intensity of their color, are not always continuous from the base to the tip of the leaf. In such cases they may end more or less abruptly at any point in the leaf, or may begin at any point above the base and terminate anywhere between this point and the tip.

CYTOLOGICAL FEATURES.—With regard to the green plants, careful cytological examination has failed to reveal any differences between their cells and those of green plants of unaffected strains.

The cells of the yellowish green plants show a series of stages in the development of chloroplasts from minute proplastids which are similar in all respects to the stages in the green plants, with the exception of the maximum size and depth of color attained. The proplastids increase in size until many of them have become well differentiated plastids with a diameter of 4.0–5.5 μ (figs. 33–41), whereas the plastids in normal green plants have a diameter of 7–8 μ; very exceptionally the latter size may be attained (fig. 41). The plastids in the pale areas of the seedling leaves are fewer in number, and the proplastids are more numerous than in the corresponding cells of a green plant (fig. 39). A study of the living cells shows a variable behavior as regards the elaboration of chlorophyll in the young seedling leaves. In the mesophyll cells most of the plastids become faintly green as soon as they become partially mature (fig. 39), while in cells near the vascular bundles plastids are often observed in which the color has become nearly as deep as in a normal mature chloroplast (fig. 41). In cells somewhat removed from the bundles all gradations between the lighter and darker shades are seen (fig. 40). In addition to the uniformly colored plastids, there are others which show an uneven outline and an irregular distribution of the pigment within them. For example, the color is often confined to one or two regions within the plastid, the limits of these regions in some cases being clearly marked and in others very vague (fig. 39). Such appearances strongly suggest degeneration or other disturbances of a serious nature. Not only young cells, but cells which are completely matured, contain proplastids in various stages of enlargement.

The plastids are much more fully developed in the yellowish green plants than in the albino seedlings of the "Mendelian white" strain, but a sufficient amount of chlorophyll for continued growth fails to be developed, and the plants die in the seedling stage. They may become slightly larger, however, than the white seedlings of the Mendelian strain. In the yellowish green plants there is little correlation between the degree of development reached by the plastids and the amount of chlorophyll elaborated. There are abundant well differentiated plastids present but only a rela-

tively small amount of chlorophyll. The failure of the plants to become green cannot be ascribed to a failure of the plastids to develop, but rather to an absence of a sufficient amount of chlorophyll.

The striped plants of this strain possess varying amounts of green and yellowish green tissue, as already pointed out. To the naked eye the boundary between the green and yellowish green areas appears to be very sharp, but when viewed with the microscope there is seen a region of transition one to several cells in width, in which are found plastids showing many intermediate sizes and depths of color, even within a single cell. Although the transition regions may vary in width, careful search has so far never failed to reveal cells which are in some degree intermediate in character. In some cases there is a single transitional cell which may contain plastids of many shades of green, whereas in other cases there may be a series of several transitional cells in each of which all or nearly all of the plastids are of one intermediate shade. Fig. 50 illustrates the condition which is most frequently found to occur in transitional regions. The cells on the left are characteristic of yellowish green tissue, those on the right are typical of green tissue, the ones between contain plastids which vary greatly, both in size and in intensity of green pigment. In the light of such facts the inapplicability of hypotheses involving a simple sorting out of plastids of two completely distinct types by successive cell divisions is clearly evident, so far as the color types in the strain of maize under consideration are concerned. To this point we shall return.

Intra-vitam stains and the use of fixing and staining reactions commonly employed have as yet given no evidence that the class of cell elements described comprise bodies of more than one kind. It is hoped that further studies may contribute something to this phase of the problem.

Summarizing, all of the plant types examined have been found to be the same as regards the cytoplasmic inclusions of their meristematic cells. Minute proplastids of the same size, shape, and general appearance have been observed in the living cells of all the chlorophyll types of maize studied. Intra-vitam stains and the fixing and staining reactions which have been used by other

workers for the study of cytoplasmic structures have given no evidence for the assumption that the group of cell elements under investigation consists of bodies belonging to more than one class. The observed differences between the various types of plants lie in the subsequent behavior of the proplastids with reference to the development of chloroplasts. From a structural standpoint the cells of all the plant types appear to be initially alike. The differences which later appear seem rather to be dependent upon the relative amount of chlorophyll developed in organs, the primordia of which are present in all cells. It is probable, therefore, that the ultimate explanation of unusual types of behavior may involve functional rather than structural differences.

Discussion

BEARING OF RESULTS ON PLASTID INHERITANCE.—The hypotheses which have been advanced to explain plastid inheritance have been based chiefly on the breeding behavior of the plants. The question remains as to how far these hypotheses will be substantiated by cytological observations. For the most part such hypotheses involve the assumption that plastids are permanent cell organs, arising only by a division of others of their kind, either while they are in the form of minute primordia or after they have reached the mature stage. The fact that proplastid-like bodies are present in the early embryonic stages, and the well substantiated evidence that mature plastids arise from bodies which cannot be distinguished from these elements, however, do not seem to constitute sufficient proof of the theory that plastids are cell organs having an unbroken continuity through all stages of the life cycle. Observations suggest that plastids may also differentiate anew at certain times in the life of the cell, which is obviously of the highest importance in connection with the problem in hand, and will be fully discussed later.

The frequent occurrence of chlorophyll abnormalities has furnished abundant material for a study of plastid inheritance. Many cases have been reported, and the breeding behavior carefully studied. The inheritance of some of the known cases is clearly Mendelian, but other types are clearly non-Mendelian. Thus the

known cases of plastid inheritance do not appear to be in the same category, and as yet have not been explained by a common well substantiated hypothesis.

Examples of chlorophyll characters which are inherited as Mendelian recessives have been described by numerous workers. Among the researches dealing with such characters may be mentioned those of EMERSON and LINDSTROM in maize, and those of BAUR, KIESSLING, TJEBBES and KOOIMAN, MIYAZAWA, CORRENS, and ALLARD on a number of other plants. The hypothesis held to account for the transmission of these characters is the same as that upon which the behavior of other Mendelian characters has been explained. Although the character in question is expressed in the plastids, the cell activities which result in the appearance of the character seem to be under the control of nuclear factors. If the character is thus under the control of a nuclear factor, it is nevertheless of importance to determine whether plastids are present in colorless cells as well as in normal ones; and also to determine to what extent the plastid is a permanent cell organ multiplying by division, especially if it is desired to gain insight into the process through which the character is brought to expression.

MILES (**47**), in a cytological investigation of albino seedlings in maize, reported that in the pure white plants no plastids could be differentiated. This does not at all correspond with the findings of the writer. Partially matured plastids are present in all of the mesophyll tissue of the plant, and in certain regions a limited number of mature functional plastids are present in some cells. Plastids, therefore, are not entirely absent, but their development is permanently retarded in the white seedlings. In the virescent plants the normal development of the plastids is interfered with only in the seedling stage.

Other chlorophyll types in maize which are Mendelian appear to be normal in the seedling stage, but the character appears in the plant as it approaches maturity. In fact there are known in maize markedly different chlorophyll types, and it seems certain that additional ones will be described. These cases are of interest cytologically in that apparently there is a wide variation in the condition of the plastids in the affected plants.

The cases in which the inheritance of plastid characters is non-Mendelian present a very complex problem. Added significance is here attached to the plastids, and workers are led to assume that the plastids themselves in the main are responsible for the inheritance of the characters which are manifested in them. As in the case of the Mendelian plastid characters, this assumption involves the question of the origin and permanence of the plastids, to be discussed later. The cases of non-Mendelian inheritance of chlorophyll variation have been classified as (1) maternal and (2) biparental.

Attention has already been called in the introduction to the well known cases of maternal inheritance reported by BAUR, CORRENS, and GREGORY, as well as to that found in maize by ANDERSON, whose results have not yet been published. CORRENS assumes that in *Mirabilis* the absence of chlorophyll is due to a cytoplasmic disease which in some way affects the plastids. Two kinds of plastids are said to be present, green and colorless ones, whose segregation during the divisions of the somatic cells is assumed to explain the presence of "checkered" leaves, as well as the complete absence of color in entire leaves and branches. BAUR offers a somewhat different explanation for similar cases. He believes that there are two kinds of plastids, diseased and normal ones, present in the mature leaf tissue, both being permanent cell organs with a definite individuality. The primordia of the two kinds of plastids are supposed to be transmitted from one generation to the next through the cytoplasm of the egg. A somatic segregation of these primordia to different cells during the growth of the plant accounts for the green and white areas of the mature plant. The hypothetical nature of this explanation is to be admitted, inasmuch as different kinds of primordia have not been demonstrated in these plants, from which it follows that the postulated segregation has not been observed. An examination of the mesophyll cells of plants produced by the maternal inheritance strain in maize fails to lend support to either of these theories. The development of the plastids from granular proplastids has been traced carefully in the living cells, and the condition of the plastids in the leaf tissue of the seedlings and mature plants has been

studied thoroughly. A variation, not only in the size of the plastids, but in the amount of color pigment as well, is characteristic of all the plant types.

Two distinct kinds of plastids, normal (green) and chlorophyll-less ones do not occur in the strains of maize studied; the green and colorless plastids observed in these strains do not represent distinct categories at all. The yellowish green plants of the maternal inheritance strain produce some plastids which have a diameter equal to that of the plastids present in normal green plants, and the intensity of the green color may equal that of green plants. In general, however, the plastids are slightly smaller and paler in color. The striped yellowish green and green plants show no sharp segregation of two distinct kinds of plastids. Cells on the border line between the two regions contain plastids of varying sizes and intensities of color. A single cell in such a region often contains plastids showing all degrees of variation found in either yellowish green or green areas. Such intermediate conditions are prevalent in the transition region between the two kinds of tissue, and at no time can there be found two distinct kinds of plastids, either in different cells or in the same cell. GREGORY made a study of the breeding behavior of a strain of *Primula sinensis* which produced chlorotic plants. The experimental results showed the character to be maternal in its inheritance. In chlorotic cells the plastids were shown to be pale yellow and smaller than in cells of the green tissue, but in any individual cell of mature tissue the plastids were found to be all alike. In young actively growing cells, however, different kinds of plastids occur in the same cell, which are similar to the two kinds found in the green and chlorotic cells of the mature leaf. GREGORY uses this as evidence that both kinds occur together in embryonic tissue, and later become segregated to different regions of the plant, and concludes that the abnormality is localized in the plastids. There is, however, another interpretation to be placed on the variation in the size and color of the plastids present in the growing cells of the leaf. In maize there can be no doubt that this condition is due to the presence of different stages in the development of plastids of one kind. The association of plastids showing a great variation in

size and color in the same cell is characteristic of embryonic cells in normal green plants. Since it was in such cells that GREGORY reported the presence of the two supposedly different kinds of plastids, this interpretation seems to be a more plausible one for his observations. Convincing cytological evidence of the occurrence of two distinct kinds of plastids and their segregation to different regions of the plant is at present lacking. In view of the condition observed in maize, it is clearly evident that visible structural differences in meristematic cells cannot be held to account for the inheritance and development of chlorophyll patterns.

All of the plant types, both the Mendelian and maternal, have the same initial cell structure, so far as observations have gone. It has not been possible to distinguish different kinds of proplastids in the youngest cells examined, that is, those of the promeristematic tissue of very young stems and the meristematic regions of embryonic leaves. The actually observed differences lie in the subsequent behavior of the proplastids with reference to the evolution of plastids.

Whether the proplastids arise by division or *de novo*, they are found to be present in the meristems of all of the chlorophyll types. The fertilized eggs of the different strains have not been examined, but if they differ as regards their visible cytoplasmic inclusions, corresponding differences would certainly be expected in the undifferentiated embryonic tissues of these strains; but careful examination of such tissues has failed to reveal any differences. As already stated, therefore, the differences in such types seem to be due to different modes of subsequent behavior on the part of proplastids in different plants and in different regions of the same plant, rather than to initial proplastid differences in the fertilized eggs of the different types. This view is supported by the occurrence of striped plants in the maternal inheritance strain, and also in the Mendelian strains which are not here reported.

It is necessary to account for different kinds of behavior on the part of proplastids which are, so far as microscopic examination shows, initially alike. Since visible bodies of more than one kind cannot be seen segregating, it might be suggested that there is an invisible structural difference in the cytoplasm of the different

plant types, or even in different parts of the same plant (striped). Thus the difference between the green and white plants in both the maternal and Mendelian categories depends upon an invisible differentiation process in the cytoplasm of the different cells, which influences the course which the proplastids take in their development, and the degree of development reached. One is then led to inquire at what stage in the life cycle this differentiation, which may be regarded as a physiological process, occurs. In the case of the maternal types, the irregular distribution of the green and white plant-producing seeds on the ear suggests that it may occur in the formation of the egg, or even earlier in the development of the ear, because of the frequent presence of irregular patches of affected seeds. The occurrence of the differentiation at some stage in embryogeny would account for the striped plants, in early division stages for the plants with large areas of the pale green tissue, and in the later stages for the plants having smaller amounts of such tissue. In the Mendelian types differentiation which is probably of a different kind must occur at sporogenesis, like any other Mendelian character. The two types of behavior, the Mendelian and maternal, are not in the same category, and as yet cannot be explained by a common well substantiated hypothesis. It is hoped that further studies of cytoplasmic structure will furnish evidence of value in the solution of the problem, and show more clearly what phases of the problem are to be definitely assigned to the physiologist.

RELATION OF PROPLASTID TO CHONDRIOSOMES.—One of the most outstanding questions arising from the present study is that regarding the relation which may exist between the proplastid of the foregoing description and the minute cell elements which other investigators have described under the name of chondriosomes, mitochondria, etc. There is great confusion at the present time concerning the nature and significance of these elements. It is plain from a survey of the very extensive literature dealing with the cell elements of this general class that the opinions of the most competent observers are in conflict on many important points. It is probable that cell inclusions of many different kinds may have been designated by the term chondriosomes, and whether

any particular body falls under this class depends upon the definition employed.

The present study has shown beyond a doubt that plastids develop from minute primordia in the different types of maize under consideration. What has so far been determined regarding the nature of these primordia and the relation which they may bear to chondriosomes must be regarded as insufficient to warrant an extended critical review of this question. It is planned to continue the investigation of this point. Nevertheless a brief comparison with the observations of other investigators who have devoted special attention to the chondriosomes may contribute something toward a solution of this puzzling problem.

The development of plastids from granular primordia has been observed by many workers, but there is a wide diversity of opinion as to the relation of the primordia to other cytoplasmic inclusions. One group of workers maintains that plastids arise from chondriosomes (mitochondria) which are permanent cell organs, and which correspond to similar bodies described in animal cells (MAXIMOW, PENSA, LEWITSKI (**41**), GUILLIERMOND, EMBERGER). GUILLIERMOND (**24–34**), as a result of many researches on plant cells, believes that plastids arise from rodlike mitochondria (chondriokonts) and differ from the latter only in size and to a certain extent in chemical constitution. TWISS (**56**) reports that in fixed and stained root tip cells of *Zea Mays* an unbroken series from globular, ellipsoid, or short rod-shaped mitochondria to mature plastids can be traced from the embryonic region backward, and concludes that mitochondria are normal constituents of the cytoplasm. He believes, however, that the evidence for the division of the mitochondria, as well as that for their function in heredity, is inadequate. EMBERGER (**18**) reported that there are two kinds of bodies (young plastids and mitochondria) present in the cells of certain plants, which differ slightly in size and staining intensity; both are to be classed under the general term mitochondria. Other workers hold that the plastids do not come from chondriosomes, but from bodies distinct from them (**48, 50, 52, 53, 54**). A third group of workers (TISCHLER, VON DERSCHAU, etc.) contend that the plastids are ultimately of nuclear origin, since they arise from chromatic

bodies extruded from the nucleus. Still others question the relationship between plastids and chondriosomes. HARPER (35) states as follows:

> None of the evidence so far adduced as to the specific genetic relationship between chondriosomes and plant plastids is in any way adequate . . . . that in certain cells the plastids can be recognized as very small cytoplasmic bodies with no starch in them was adequately established by SCHIMPER, but that the plastid bodies necessarily and regularly arise from the chondriosomes it seems to me is by no means proved by such crude and diagrammatic figures and seriations as those so far presented.

Views similar to this have been held by many other workers (LUNDEGARDH 46, LÖWSCHIN 44, 45, etc.).

From this brief review it is evident that there are, at present, many conflicting views in regard to the class of cell inclusions known as chondriosomes, and the relationship which exists between plastids in their initial stages and these bodies. It is furthermore apparent that this diversity of opinion is in some measure a consequence of the lack of agreement as to what constitutes permanent cytoplasmic organs, and as to what bodies should be included in the class "chondriosomes." The problem has been complicated further by the fact that certain observers have used a great variety of fixatives, which in some cases appear to have preserved the bodies in question with relatively little alteration, while in other cases artifacts have been produced which have led to various misconceptions.

No reader of chondriosome literature can fail to recognize the fact that much of the obscurity which surrounds the subject is due to the lack of uniformity in the application by various workers of the term chondriosome. By some the term has been applied very widely to a considerable variety of minute cell inclusions, some of which are seen to develop into plastids. Others are inclined to make a distinction between chondriosomes on the one hand and the primordia of plastids on the other. Still others further restrict the application of the term chondriosome, using it only with reference to a concrete class of cell inclusions which can be shown to have a particular histochemical constitution. Thus COWDRY (10) defines chondriosomes primarily as "substances which occur in the form of granules, rods, or filaments in almost all

living cells, which react positively to Janus green, and which, by their solubilities and staining reactions, resemble phospholipins and, to a lesser extent, albumins."

Noteworthy in this connection are the statements made by MOTTIER (50) as a result of his studies on the origin of plastids. In his first contribution on this subject leucoplasts and chloroplasts were shown to be derived from minute "plastid primordia." Other minute bodies present in all cells but not developing into plastids were referred to as chondriosomes. In a more recent paper (51), in which the development of protein bodies (aleurone grains) from minute primordia is reported in the endosperm cells of *Zea Mays* and other plants, emphasis is no longer placed upon this distinction between primordia and chondriosomes. It is stated that the latter, because of their obscure rôle, were previously called chondriosomes mainly for convenience. In view of the fact that the terms mitochondria and chondriosome have been so loosely applied to plastid primordia and many other cytoplasmic inclusions as well, MOTTIER does not use these terms in his most recent description, and even suggests that much ambiguity might be avoided by dropping the terms entirely.

The present writer has also felt that much confusion might result from the use of the term chondriosome in his description. In the material studied there appears to be but one class of cytoplasmic granules, as already stated, these granules representing the early stages in the development of plastids. In order to avoid any implication as to the ultimate nature of these bodies and their relation to other cytoplasmic inclusions described by various workers, they have been designated in the present paper by the term proplastid. Whether or not the proplastid is a chondriosome is a question which can be answered only when the histochemical nature and developmental history of cytoplasmic inclusions have become better known, and when a definite and uniform terminology has been settled upon. The proplastids can be readily observed in the living cells. They are clearly visible in the cells of the promeristematic tissue of young stems and embryonic leaves, and their activities in these cells and their subsequent behavior in older cells have been closely followed through all stages of their develop-

ment. Although they can be so studied in the living cells, it is nevertheless necessary to employ methods which will not only permit one to view the living cell for a moment in the "living" condition, but which will allow continued observation of the actively functioning cell over a considerable period of time, thus avoiding alteration in the cytoplasm which may be brought about very quickly when the living tissue is improperly handled.

The proplastids appear in their initial stages as refringent globules of varying size, always sharply distinct from the ground substance of the cytoplasm. They are constantly changing their position in the embryonic cells, being often carried about rapidly by the streaming cytoplasm. This phenomenon makes it appear certain that even the most minute of the proplastids are special structures distinct from the principal mass of cytoplasm in which they lie. Particular attention has been devoted to these minute proplastids which lie just within the lower limit of the range of visibility. They have been examined with lenses of very high resolving power (Zeiss apochromatic objective, 2 mm. N.A. 1.40 and compensating ocular 6), and their activity has been repeatedly watched very closely. Very important questions concerning the ultimate nature and significance of proplastids are here involved, and will be discussed in another connection later. The subsequent enlargement of the proplastids to form chloroplasts can be traced step by step throughout their course of development, abundant material being readily obtainable from young seedlings.

Although comparatively few investigators have based their interpretations mainly on the study of living cells, they agree with the present writer in reporting the presence of such minute bodies in the living meristematic cell. In older cells, however, many phenomena have been described which the present writer has not observed, although this may be due in part to the fact that this study has been limited largely to mesophyll cells. The few observations made on the cells of other tissues indicate that additional types of cell elements and modes of behavior may be present there. For example, conditions similar to those figured by GUILLIERMOND in epidermal cells of flower petals and other floral organs have been seen in the epidermal cells of maize, but not in the mesophyll cells.

Intra-vitam stains, a number of which have been employed, have unexpectedly failed to give a definite reaction to the proplastids. Such stains as Janus green B, violet of Dahlia, neutral red, etc., have given uncertain results. In some cases the proplastids have appeared to be faintly stained by Janus green B, but in no case has a strong staining reaction been observed, neither has it been found possible to differentiate different kinds of minute bodies in the cytoplasm of the cell. COWDRY (11) reported that plant mitochondria are stained with Janus green B, but that it is much more difficult to obtain a good stain in plant cells than in animal cells because of their well developed cellulose walls. A similar conclusion has been reached by other plant workers.

Chondriosomes in both plant and animal cells were first observed in fixed and stained preparations, and the lack of uniformity in the results of early workers may have been largely due to the use of different fixatives and alterations resulting from poor fixation, as has been pointed out above. The special methods of fixation and staining commonly used in a study of the cytoplasm have been fully described in recent literature (COWDRY 11, GUILLIERMOND 31, KINGSBURY 39). In general chondriosomes have been found to be preserved by potassium bichromate, chromic acid, neutral formalin, and osmic acid, and these are the chief ingredients of the fixatives used for their study. Such substances as alcohol, ether, chloroform, and acetic acid dissolve the chondriosomes or produce profound changes in their shape, etc.

The methods of fixation ordinarily employed often appear to preserve quite faithfully the structures in question, but direct observation on the living cell is unquestionably of far greater value, since it obviates the possibility of misinterpretation to a very large extent. Furthermore, in tissues which are favorable for a study of the living cells the cytoplasmic inclusions are sharply delimited, and actually are more easily observed than in fixed and stained cells. Conflicting results have been obtained by those who have attempted to demonstrate the existence of different kinds of cytoplasmic bodies by the use of various fixing and staining methods. The use of such methods in this study has failed to furnish convincing evidence for the presence of more than one type of proplastid in the initial stages.

Chondriosomes of widely different shapes have been observed, the commonest forms being the granular mitochondria and the rod or thread-shaped chondriokonts. These and other types are not looked upon as belonging to distinct classes by plant cytologists, but rather as transitional stages between granular elements and structures such as chloroplasts, chromoplasts, elaioplasts, etc., concerned in the elaborative functions of the cell. In animal cells the chondriosomes have been seen to undergo marked changes in shape (LEWIS and LEWIS **40**), but it may be that such changes are more gradual in plants. The proplastids in maize are of a single uniform shape in the mesophyll cells, being granular in the early stages and spherical or ovoid in later ones. Rodlike elements are present in the epidermal cells, but not in the cells associated with chloroplast formation. This variation in the size, and the different reactions to certain methods of fixation and staining have been used by workers as evidence for the existence of different kinds of elements which can be distinguished in embryonic cells and which perform certain definite functions. Prominent workers maintain that plastid primordia can be distinguished from mitochondria at all stages in their development (RUDOLPH, MOTTIER, DANGEARD). This conclusion is based chiefly on size differences and on slight variations in fixing and staining reactions. This contention has been supported by the statement that unaltered mitochondria are present with the chloroplasts in mature cells, the inference being that all the plastid primordia have developed into plastids, while the mitochondria remained unchanged. The statements of MOTTIER already cited are noteworthy in this connection. The present study has shown that in maize these small bodies in the mature cells are without doubt to be regarded as plastids which are for the most part in a very retarded state of development. A sufficient number of intermediate stages between the smallest ones and mature plastids are present to make it appear certain that these bodies do not represent a distinct category unlike the proplastids of meristematic cells. This question of the possible existence of more than one type of initial granule has received critical attention throughout the present study, and inasmuch as the origin of the chloroplasts can be traced backward through

successively smaller stages to proplastids which lie just within the lower limit of visibility, there appears to be no justification for the view that more than one kind of initial granule is present in the meristematic cell. It is only through a study of these minute initial stages that many of the principal questions involving chondriosomes and plastids can be answered. These earliest stages seem to have received but scant attention from previous workers. It is to be emphasized that the evidence at hand leads directly to the conclusion that the various structures observed in later stages (green and white plastids) have arisen by a process of differentiation from bodies of one kind, rather than from bodies initially unlike. The cause of such a difference in behavior on the part of the primordia can only be conjectured.

INDIVIDUALITY OF PLASTIDS.—The proplastids have been found to be a constant feature of cytoplasmic organization in maize, and the question arises as to the origin of these bodies. The definiteness of their behavior in relation to the evolution of chloroplasts indicates that they are at least concerned in the elaboration of certain products of cell activity. Whether or not they may also be found to be associated with other vital functions remains as a problem for further research.

Plastid primordia have been considered by previous workers as essential constituents of the cytoplasm which retain their individuality and persist throughout the life cycle (FORENBACHER **21**, PENSA, CAVERS **5**, GUILLIERMOND, MOTTIER, EMBERGER). The evidence from primitive plants (ALLEN, SAPEHIN, SCHERRER), in which well developed plastids are present throughout the life cycle, may be considered as evidence favoring this view. The occurrence of division stages in partially developed chloroplasts suggests also that the smaller bodies may divide, but my observations indicate rather that such an assumption is hardly warranted. Appearances have frequently been found which suggest division stages, but definite proof of division is very difficult to obtain. The fact that a frequent association and subsequent separation of the proplastids occur in embryonic cells, as herein described, renders it unsafe to draw conclusions on this point. Even if it were shown that these minute bodies multiply by

division only, it would still remain to be shown whether or not the same would hold true for stages before the bodies have become large enough to be seen. The writer has repeatedly satisfied himself that the series of stages observed actually grades off to the lower limit of visibility.

In view of the uncertainty regarding the mode of plastid origin in young meristematic cells, it seems probable that the question of the genetic continuity of plastids would not be answered with finality by a study of the gametes by methods at our command. In the present research the gametes have not been observed. If it were found that the fertilized egg contains no visible proplastids, the presumption would be strong in favor of the *de novo* origin of these bodies in embryonic cells. It is conceivable, however, that proplastids too minute to be observed might nevertheless be present and be multiplying only by division. On the other hand, if the fertilized eggs were found to contain proplastids, the mode of their origin and multiplication would probably be no easier to determine than in older meristematic cells which have been studied and described. It therefore seems that the impossibility of determining the mode of proplastid origin in meristematic cells requires that the question of the continuity of plastids shall remain an open one, regardless of any condition which one might expect to observe in the gametes. In view of these facts, explanation of plastid behavior which are based on the assumption of a complete individuality on the part of these organs are unsound. The only other alternative is that of *de novo* origin. The conception of the cytoplasm as a substance in which certain processes become localized, with the accompanying new differentiation of regions which are cell organs, has been furnished with a clear statement by HARPER (**35**), as follows:

> What seems to me the most important advance in our knowledge of cell architecture has been in the direction of the recognition of localized spatially differentiated regions of the cell body in which certain processes occur . . . . the plastid is to be regarded as a region of the protoplasmic complex rather than a differentiated and definitely delimited body . . . . cytologically the chloroplast is perhaps little more than an area of the cytoplasm impregnated or infiltrated with chlorophyll.

The possibility of the *de novo* origin of cell organs is one which should be more generally recognized. With regard to the plastids it must be admitted that the evidence so far obtained by cytologists does not permit a definite decision in favor of either the *de novo* or the individuality theory. The exact manner in which plastids originate in the cell is obviously of the greatest importance to those who are searching for the explanation of the behavior of inherited characters which manifest themselves in these organs. If plastids are not passed on as permanent individuals, some other explanation must be offered for their repeated appearance and regular behavior in successive generations.

Summary

1. All the chlorophyll types examined were found to contain the same initial cell structure, minute "proplastids" of the same size and general appearance being present in every type.

2. In normal green plants the proplastid first appears in the cell as a minute granule at the limit of visibility, gradually enlarging and developing chlorophyll until it becomes a mature chloroplast. In plants of the other chlorophyll types studied (Mendelian white, Mendelian virescent, and the maternal inheritance strain) the unusual characters of the plants are due to the failure of the proplastids initially present to develop into plastids with the normal size, or color, or both.

3. The green and colorless plastids found in different plants or in different portions of the same plant do not represent two fundamentally distinct types, but are rather to be regarded as the end members of a continuous series which comprises also all intermediate conditions. No cytological evidence was found favoring the view that the primordia from which the variously developed plastids arise are of more than one kind.

4. Partially developed and fully matured plastids may be seen multiplying by division, but when first visible the proplastid is so minute that it is impossible to determine the mode of its origin. The division of partially mature and mature plastids emphasizes the fact that they have a distinct individuality at such stages;

but in view of the obscurity which surrounds the origin of the minute primordia from which the plastids first appearing in the embryonic cells arise, the question regarding the extent to which the plastids are to be considered permanent cell organs with an unbroken genetic continuity throughout the life cycle must remain an open one.

5. In the case of those strains in which the inherited characters are transmitted according to Mendelian rules, it is inferred that the behavior of the proplastid is at least in part under the control of the nuclear mechanism. In those strains in which the inheritance of the unusual characters is non-Mendelian (maternal strains), it is very probable that an explanation of another kind will be found necessary.

This investigation was carried on under the direction of Professor LESTER W. SHARP, to whom the writer wishes to express his sincere thanks and appreciation.

CORNELL UNIVERSITY
ITHACA, N.Y.

LITERATURE CITED

1. ALLARD, H. A., The Mendelian behavior of *aurea* character in a cross between two varieties of *Nicotiana rustica*. Amer. Nat. **53**:234–238. 1919.
2. BAUR, E., Des Wesen und die Erblichkeitsverhältnisse der "Varietates Albomarginatae hort." von *Pelargonium zonale*. Zeit. Ind. Abst. Vererb. **1**:330–351. *figs. 20*. 1909.
3. ———, Untersuchungen über der Vererbung von Chromatophoren Merkmale bei *Melandrium*, *Antirrhinum*, und *Aquilegia*. *Ibid.* **4**:81–102. 1910.
4. ———, Mutationen von *Antirrhinum majus*. *Ibid.* **19**:177–193. 1918.
5. CAVERS, F., Chondriosomes (mitochondria) and their significance. New Phytol. **13**:96–106. 1914.
6. CORRENS, C., Vererbungsversuche mit blass (gelb) grünen und buntblätterigen Sippen bei *Mirabilis jalapa*, *Urtica pilulifera*, und *Lunaria annua*. Zeit. Ind. Abst. Vererb. **1**:291–329. *figs. 2*. 1909.
7. ———, Zur Kenntniss einfacher Mendelnder Bastarde. II. *Mirabilis jalapa xantha*, und ihre Bastarde. III. *Urtica urens peraurea*. Sitzber. K. Preuss. Akad. Wiss. 221–268. 1918.
8. ———, Vererbungsversuche mit buntblätterige Sippen. I. *Capsella Bursa-pastoris*. Sitzber. Akad. Wiss. Wien **34**:585–610. 1919.

9. COWDRY, E. V., The vital staining of mitochondria with Janus green and diethylsafranin in human blood cells. Internat. Monatschr. Anat. Physiol. **31**:267–286. 1914.
10. ———, The general functional significance of mitochondria. Amer. Jour. Anat. **19**:423–446. 1916.
11. COWDRY, N. H., A comparison of mitochondria in plant and animal cells. Biol. Bull. **33**:196–228. *figs. 26.* 1917.
12. ———, Experimental studies on mitochondria in plant cells. *Ibid.* **39**: 188–206. *pls. 3.* 1920.
13. DANGEARD, P. A., Sur la distinction du chondriome des auteurs en vacuome, plastidome, et spherome. Compt. Rend. Akad. Sci. Paris **169**:1005–1010. 1919.
14. ———, La structure de la cellule végétale et son metabolisme. *Ibid.* **170**: 709–714. 1920.
15. ———, Vacuome, plastidome, et sphérome dans l'*Asparagus verticillatus.* Compt. Rend. Akad. Sci. Paris **171**:69–74. 1920.
16. ———, La structure de la cellule végétale dans ses rapports avec la théorie du chondriome. *Ibid.* **173**:121–123. 1921.
17. VON DERSCHAU, M., Zum Chromatindualismus der Pflanzenzelle. Arch. Zellf. **12**:220–240. *pl. 77.* 1914.
18. EMBERGER, L., Évolution du chondriome chez les cryptogames vasculaires. Compt. Rend. Acad. Sci. Paris **170**:282–284. 1920.
19. ———, Évolution du chondriome dans la formation du sporange chez les fougéres. *Ibid.* **170**:469–471. *figs. 7.* 1920.
20. EMERSON, R. A., The inheritance of certain forms of chorophyll reduction in corn leaves. Nebraska Agric. Exp. Sta., Ann. Report **25**:89–105. 1912.
21. FORENBACHER, A., Die Chondriosomen als Chromatophorenbildner. Ber. Deutsch. Bot. Gesells. **29**:648–660. *pl. 25.* 1911.
22. GERNERT, W. B., The analysis of characters in corn and their behavior in transmission. Univ. Illinois Thesis. 1912.
23. GREGORY, R. P., On variation in *Primula sinensis.* Jour. Genetics **4**:305–321. *pls. 9, 10.* 1915.
24. GUILLIERMOND, A., Recherches cytologiques sur le mode de formation de l'amidon et sur les plastes végétaux. Arch. Anat. Micr. **14**:309–428. *pls. 13–18.* 1912.
25. ———, État actuel de la question de l'évolution et du rôle physiologique des mitochondries. Rev. Gen. Bot. **26**:129–149; 182–210. *figs. 16.* 1914.
26. ———, Nouvelles observations vitales sur le chondriome des cellules épidermiques de la fleur d'*Iris germanica.* Compt. Rend. Soc. Biol. Paris **67**:241–249. 1915.
27. ———, Observations vitales sur le chondriome de la fleur de tulipe. Compt. Rend. Acad. Sci. Paris **164**:407–409. 1917.
28. ———, Sur les alterations et les caractères du chondriome dans les cellules épidermique de la fleur de tulipe. *Ibid.* **164**:609–612. 1917.

29. GUILLIERMOND, A., Sur la metachromatine et les composes de la cellule végétale. *Ibid.* **166**:958–960. 1918.

30. ———, Sur l'origine mitochondriale des plastides. *Ibid.* **167**:430–433. 1918.

31. ———, Observations vitales sur le chondriome des végétaux et recherches sur l'origine des chromoplastides et le mode de formation des pigments xanthophylliens et carotiniens. Rev. Gen. Bot. **31**:372–413; 446–508; 532–603; 635–770. *pls. 60. figs. 35.* 1919.

32. ———, Sur les éléments figures du cytoplasme. Compt. Rend. Acad. Sci. Paris **170**:612–615. *figs. 5.* 1920.

33. ———, Nouvelles recherches sur l'appareil vacuolaire dans les végétaux. *Ibid.* **171**:1071–1074. *figs. 25.* 1920.

34. ———, Sur les microsomes et les formations lipoides de la cellule végétale. *Ibid.* **172**:1676–1678. 1921.

35. HARPER, R. A., The structure of protoplasm. Amer. Jour. Bot. **6**:273–300. 1919.

36. IKENO, S., Studies on the hybrids of *Capsicum annuum.* II. On some variegated races. Jour. Genetics **6**:201–229. 1917.

37. KAJANUS, B., Über eine konstant gelbbunte Pisum-Rasse. Bot. Notiser. 83–84. 1918.

38. KIESSLING, L., Einige besondere Fälle von Chlorophylldefecten Gersten. Zeit. Ind. Abst. Vererb. **19**:160–176. 1918.

39. KINGSBURY, B. F., Cytoplasmic fixation. Anat. Record **6**:39–52. 1912.

40. LEWIS, M. R., and LEWIS, W. H., Mitochondria (and other cytoplasmic inclusions) in tissue cultures. Amer. Jour. Anat. **17**:339–401. *figs. 26.* 1915.

41. LEWITSKI, G., Über Chondriosomen in pflanzlichen Zellen. Ber. Deutsch. Bot. Gesells. **28**:538–546. *pl. 17.* 1910.

42. ———, Die Chloroplastenanlagen in lebenden und fixierten Zellen von *Elodea canadensis* Rish. *Ibid.* **29**:697–703. *pl. 28.* 1911.

43. LINDSTROM, E. V., Chlorophyll inheritance in maize. Cornell Univ. Exp. Sta. Memoir **13**:1–68. 1918.

44. LÖWSCHIN, A. M., "Myelinformen" und Chondriosomen. Ber. Deutsch. Bot. Gesells. **31**:203–209. 1913.

45. ———, Vergleichende experimental-cytologische Untersuchungen über Mitochondrien in Blättern der höheren Pflanzen. (Voll. Mitt.) *Ibid.* **32**:266–270. *pl. 5.* 1914.

46. LUNDEGARDH, H., Ein Beitrag zur Kritik zweier Vererbungshypothesen. Jahrb. Wiss. Bot. **48**:285–378. 1910.

47. MILES, F. C., A genetic and cytological study of certain types of albinism in maize. Jour. Genetics **4**:193–214. 1915.

48. MEYER, A., Bemerkungen zu G. Lewitski: Über die Chondriosomen in pflanzlichen Zellen. Ber. Deutsch. Bot. Gesells. **29**:158–160. 1911.

49. MIYAZAWA, B., Studies of inheritance in the Japanese *Convovulus.* Jour. Genetics **8**:59–83. 1918.

50. MOTTIER, D. M., Chondriosomes and the primordia of chloroplasts and leucoplasts. Ann. Botany **32**:91–114. *pl. 1.* 1918.

51. ———, On certain plastids, with special reference to the protein bodies of *Zea*, *Ricinus*, and *Conopholis*. *Ibid.* **35**:349–365. *pl. 15.* 1921.

52. RUDOLPH, K., Chondriosomen und Chromatophoren. Beitrag zur Kritik der Chondriosomentheorien. Ber. Deutsch. Bot. Gesells. **30**:605–629. *pl. 18. fig. 1.* 1912.

53. SAPEHIN, A. A., Untersuchungen über die Individualität der Plastide. Arch. Zellf. **13**:319–398. *pls. 10–26.* 1915.

54. SCHERRER, A., Untersuchungen über Bau und Vermehrung der Chromatophoren und das Vorkommen von Chondriosomen bei *Anthoceros*. Flora **107**:1–56. *pls. 1–3.* 1914.

55. TJEBBES, K., and KOOIMAN, H. N., Erfelijkheidsonderzockingen bij boonen III. Albinisme. Genetica **1**:532–538. 1919.

56. TWISS, W. C., A study of plastids and mitochondria in *Preissia* and corn. Amer. Jour. Bot. **6**:217–234. *pls. 23–24.* 1919.

EXPLANATION OF PLATES XI–XVI

All figures were drawn at the level of the table with the aid of an Abbé camera lucida under a Spencer objective, 1.8 mm., N.A. 1.25, with compensating ocular 18. They have been reduced approximately one-third and show a magnification of about 2250 diameters. Observations of critical stages were made with a Zeiss apochromatic objective, 2 mm., N.A. 1.40, with compensating ocular 6.

PLATE XI

Normal green plant

Except where noted, drawings are of living cells of subepidermal tissue from mesophyll region of leaf, or of meristematic cells which will later become part of mesophyll tissue.

FIG. 1.—From promeristematic region of germinating seed.

FIG. 2.—From tip of first leaf bud formed from apex of stem; median section.

FIGS. 3–6.—Developmental stages from meristematic tips of successively older embryonic leaves; figs. 3–5, median sections; fig. 6, section through cytoplasmic layer just beneath cell wall; largest proplastids in fig. 6 faintly green.

FIGS. 7, 8.—From 16 mm. embryonic leaf just before it emerges from sheath, showing cytoplasmic layer beneath cell wall in face view.

FIG. 7.—Cell near tip, green color appearing in proplastids.

FIG. 8.—Cell somewhat farther back from tip, proplastids more fully developed.

FIG. 9.—From mesophyll tissue of fully developed seedling leaf, plastids bright green.

PLATE XII

Mendelian white seedling

Amount of green color present in chloroplasts after they have reached a size of 2.5 μ indicated by depth of shading; stages represented in this series taken from a 3-inch seedling about six days after germination.

FIGS. 10–12.—Successively older stages in development of proplastids in undifferentiated meristematic tissue; cells in median section; fig. 10, promeristematic cell from stem tip; fig. 11, from tip of leaf bud recently formed from apex of stem; fig. 12, from tip of 18 mm. embryonic leaf.

FIGS. 13–16.—From different regions of 35 mm. leaf just before emerging from enveloping leaves; fig. 13, undifferentiated cell near tip (note abnormal appearance of some of proplastids); irregular shape and presence of darker regions within proplastids characteristic of certain ones; figs. 14, 15, cells 3–5 mm. from tip; fig. 14, green color entirely absent in all proplastids; fig. 15, faint greenish color in one proplastid; others show conditions suggesting degeneration; fig. 16, typical mesophyll cell of seedling leaf; slight trace of yellowish green color in some of proplastids; others almost colorless or containing irregular opaque masses.

FIGS. 17–19.—Cells from tip of unfolding seedling leaf.

FIG. 17.—Undifferentiated cell at tip of leaf; largest proplastids faintly green.

FIG. 18.—Cell 3 mm. from tip; larger proplastids (plastids) clearly green.

FIG. 19.—Mesophyll cell 4.5 mm. from tip; plastids bright green.

PLATE XIII

Mendelian virescent

Depth of shading indicates intensity of green color in later stages of plastid development; material of same age as in the case of Mendelian white.

FIGS. 20–23.—From promeristematic and embryonic leaf tissue corresponding to similar early stages in green plant.

FIGS. 24–26, 32.—From meristematic undifferentiated regions of successively older leaf tips; figs. 24–26, before exposure to sunlight; fig. 32, after exposure to sunlight.

FIGS. 27–29.—Cells from pale green area of seedling leaf.

FIG. 27.—Mesophyll cell between vascular bundles.

FIG. 28.—Mesophyll cell nearer vascular bundle.

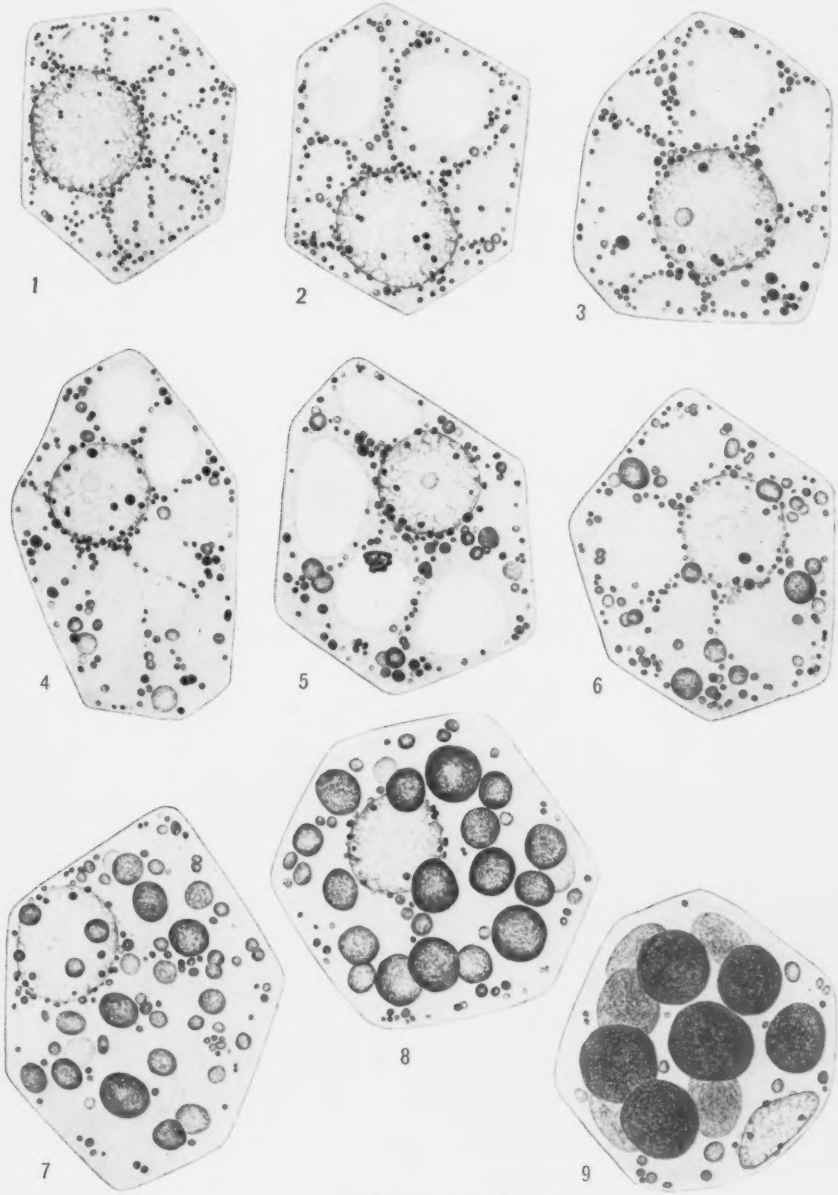
FIG. 29.—Cell lying next to vascular bundle.

FIGS. 30–32.—From transition region between pale green and green areas of seedling leaf.

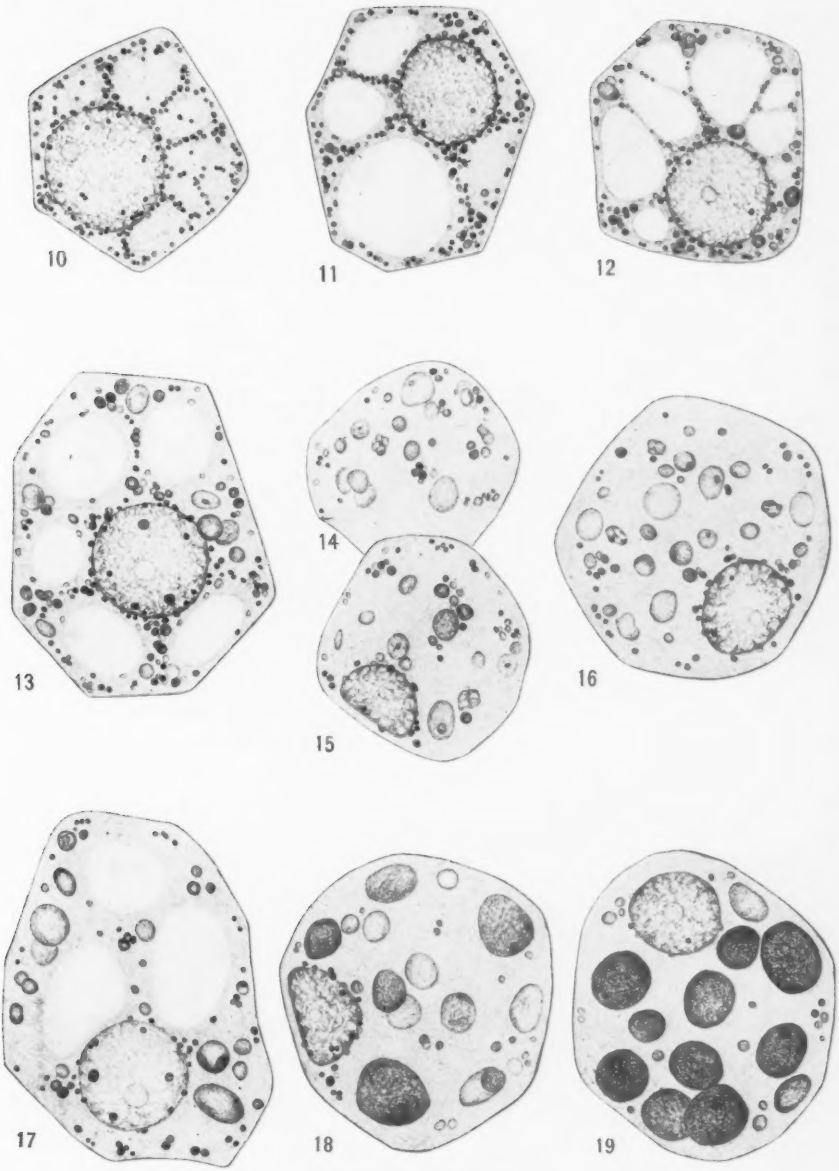
FIG. 30.—Mesophyll cell from slightly green region.

FIG. 31.—From deeper green region.

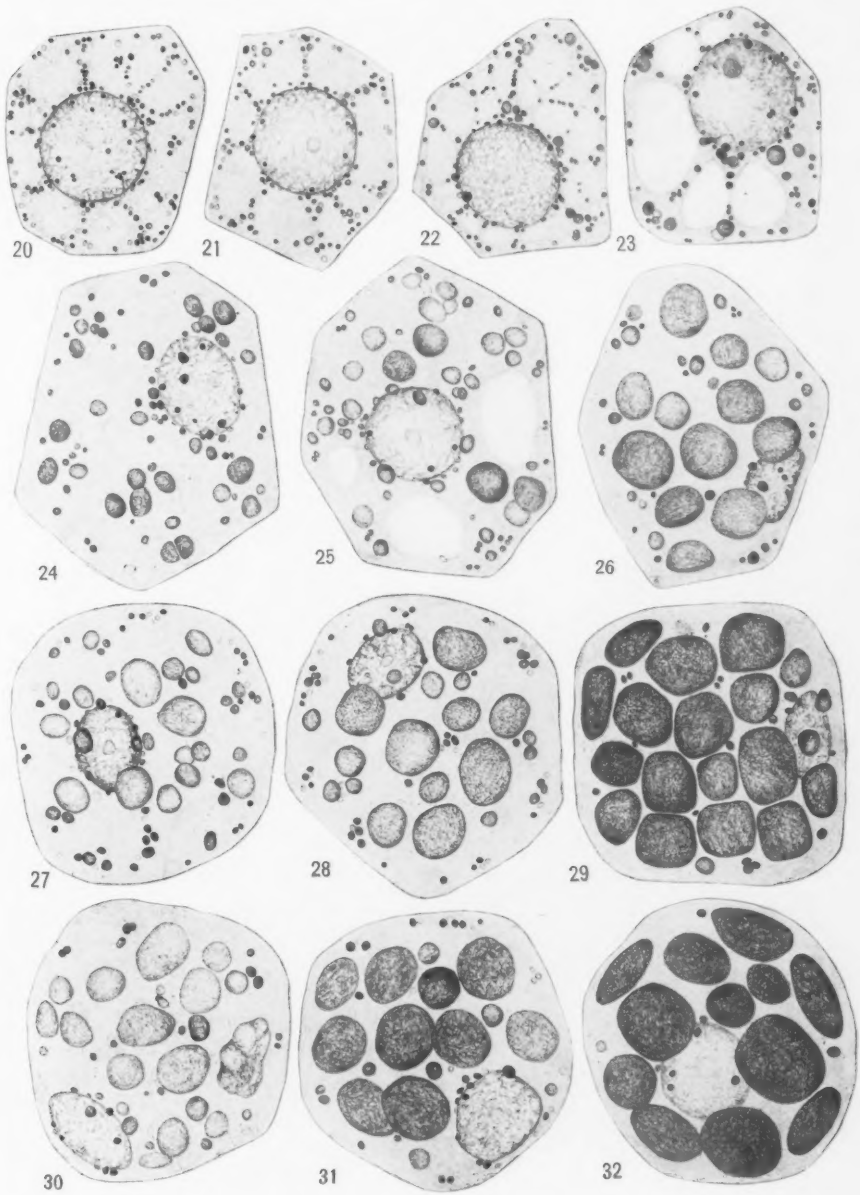
FIG. 32.—Cell from apex of green leaf.



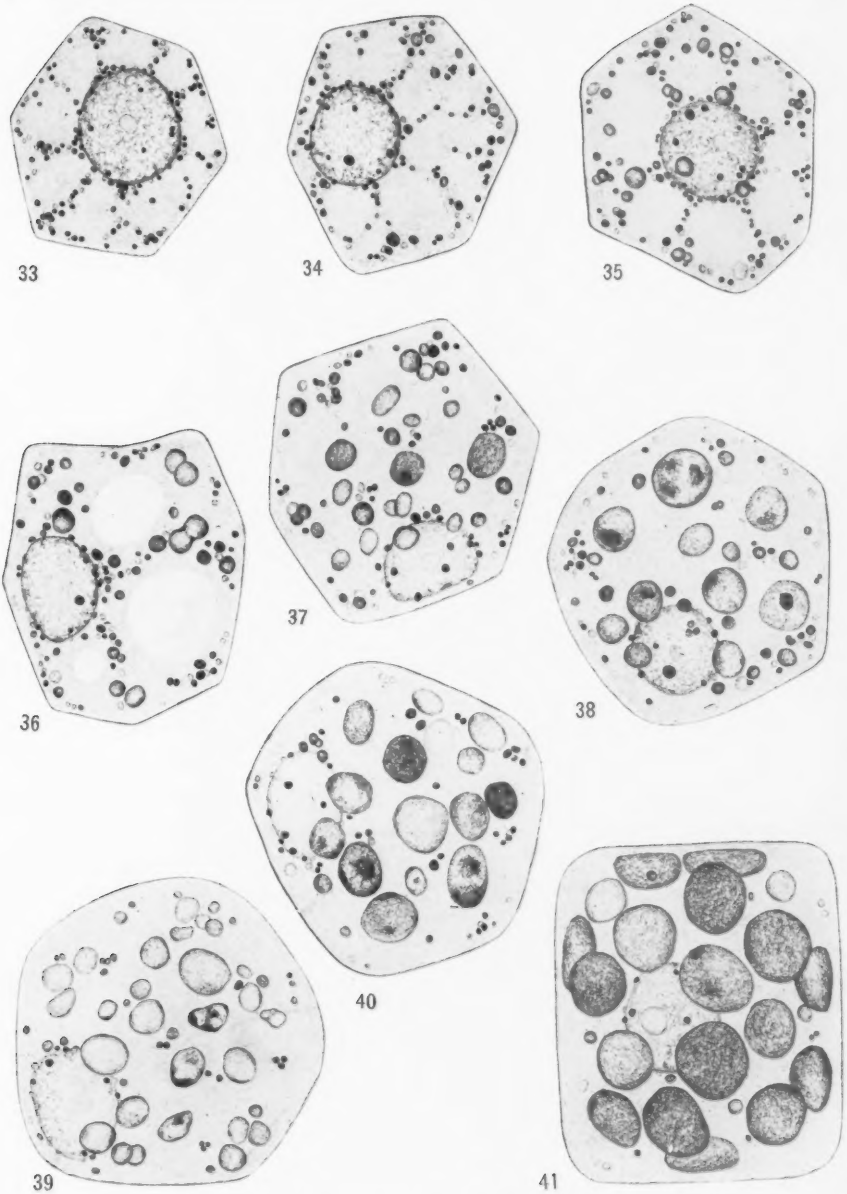
RANDOLPH on MAIZE



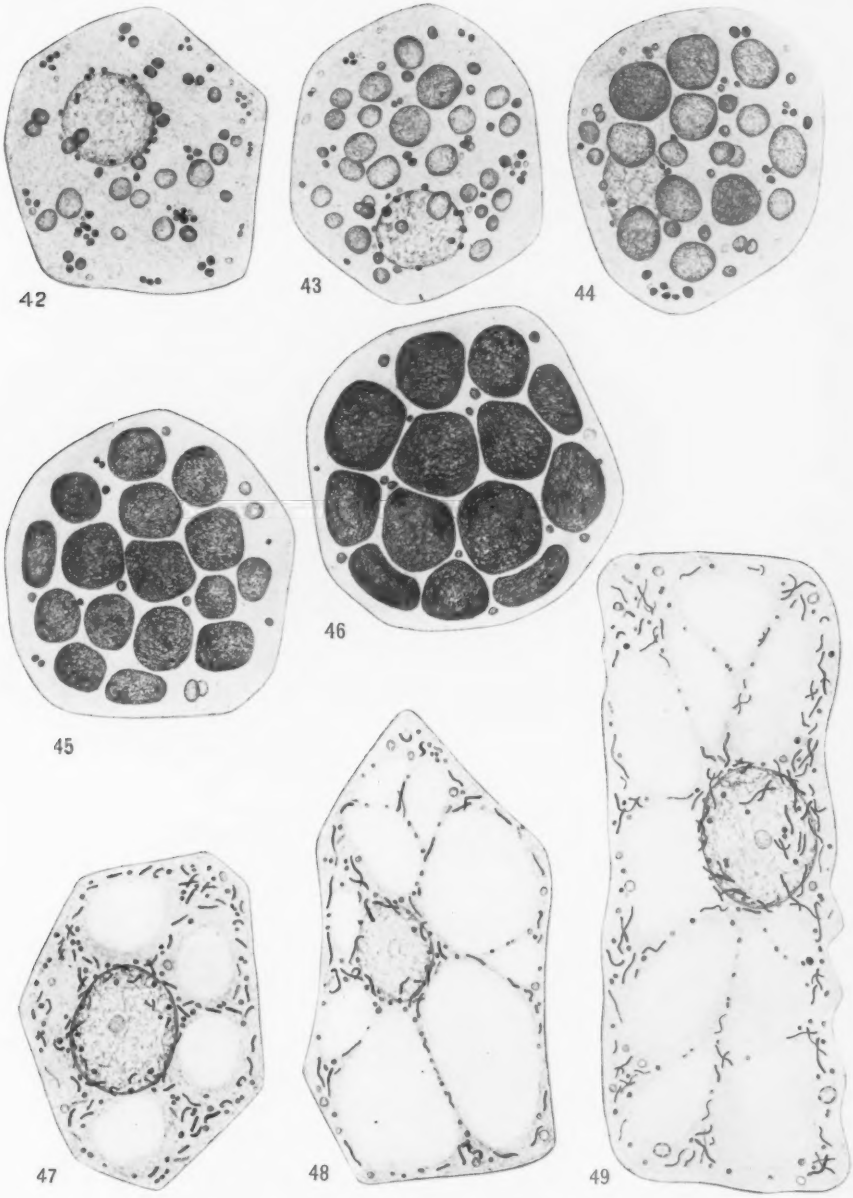
RANDOLPH on MAIZE



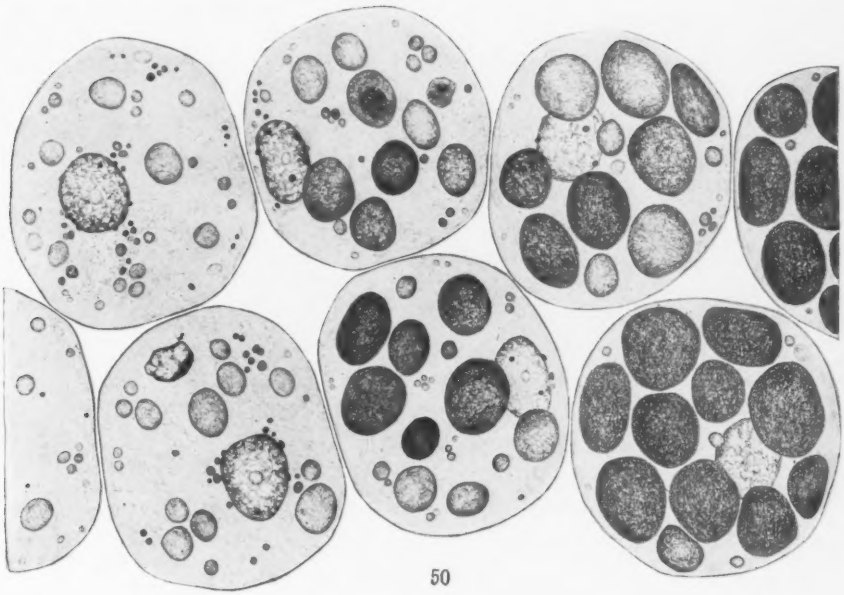
RANDOLPH on MAIZE



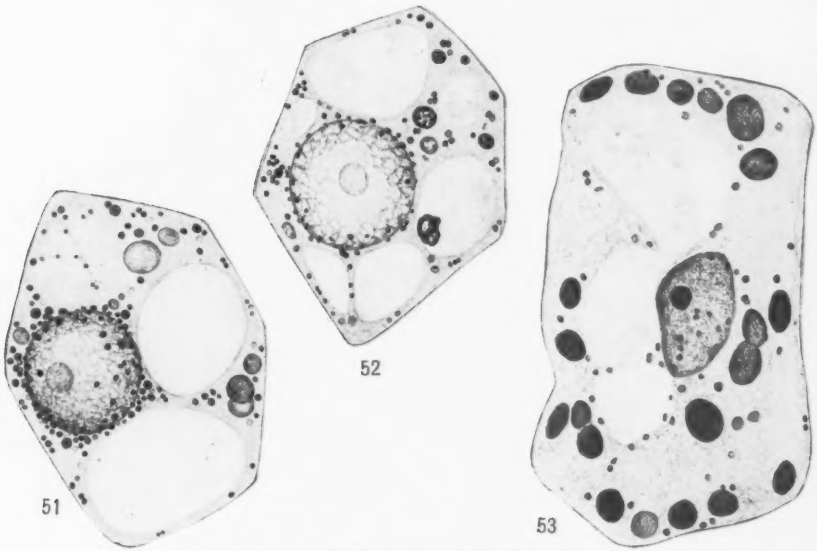
RANDOLPH on MAIZE



RANDOLPH on MAIZE



50



51 52 53

RANDOLPH on MAIZE

PLATE XIV

Maternal inheritance strain

Material from uniformly yellowish green 3-inch seedling; amount of green color indicated by depth of shading.

FIGS. 33–38.—Showing development of plastids in undifferentiated apical regions of successively older seedling leaves.

FIG. 33.—Promeristematic region.

FIG. 34.—From first leaf bud.

FIG. 35.—From leaf bud 4 mm. long.

FIG. 36.—From tip of embryonic leaf 20 mm. long.

FIG. 37.—From tip of embryonic leaf 40 mm. long.

FIG. 38.—From tip of leaf about to emerge from sheath.

FIGS. 39–41.—From mesophyll tissue of 3-inch seedling leaf; fig. 39, cell between vascular bundles; fig. 40, cell nearer vascular bundle; fig. 41, cell adjacent to vascular bundle.

PLATE XV

FIGS. 42–46.—From fully developed mesophyll leaf tissue of mature normal green plant; amount of green color indicated by depth of shading.

FIG. 42.—Cell from region of leaf blade inclosed in enveloping leaves.

FIGS. 43–45.—Stages occurring in mesophyll tissue between unexposed and exposed regions of same leaf.

FIG. 46.—Mesophyll cell from exposed region of fully developed leaf.

FIGS. 47–49.—Epidermal cells of seedling leaf tissue of 3-inch seedling: fig. 47, cell near apex of 40 mm. embryonic leaf; fig. 48, cell from same leaf, but slightly farther from tip; fig. 49, cell of 40 mm. embryonic leaf blade.

PLATE XVI

FIG. 50.—From striped plant of maternal inheritance strain, showing cells from transitional region between yellowish green stripe and green stripe; depth of shading indicates amount of green color present.

FIG. 51.—From 35 mm. embryonic leaf of a normal green 3-inch seedling, showing grouping of many proplastids about nucleus.

FIG. 52.—From same tissue as fig. 51, showing effect of osmic acid (1 per cent aqueous solution) fixation on proplastids.

FIG. 53.—From young leaf tissue after Benda's fixation and iron-haematoxylin staining.

CULTIVATION OF EXCISED ROOT TIPS AND STEM TIPS UNDER STERILE CONDITIONS[1]

WILLIAM J. ROBBINS

(WITH FOUR FIGURES)

The growth of higher plants under sterile conditions is a necessary procedure in the investigation of problems involving the direct use by higher plants of organic or inorganic substances which may be altered by bacterial action. Failure to observe sterile conditions throws doubt on the conclusions drawn from the results secured in any experiment where the direct use by plants of an organic compound and some inorganic substances such as ammonium salts or nitrates is investigated.

As indicated by WILSON (15), the methods which have been used to grow plants under sterile conditions have either attempted to grow the entire plant under sterile conditions, or to keep that part of the plant which is of importance in the special investigation in a sterile environment. LUTZ, LAURENT, LEFEVRE, MOLLIARD, GRAFE, RAVIN, KNUDSON (11), BRANNON (4), and others have described methods of cultivating entire plants under sterile conditions. MAZÉ and PERRIER (12), SHULOW, HUTCHINSON and MILLER (9), and WILSON (15) have described in some detail methods by which higher plants may be grown with their tops exposed to the normal aerial environment. MAZÉ and WILSON grew corn plants to maturity with the root systems in sterile water cultures. Isolated and mature plant embryos have been cultivated under non-sterile conditions for longer or shorter periods by BROWN and MORRIS, ANDRONESCU (2), URBAIN (16), and others. KNUDSON cultivated corn embryos, and BUCKNER and KASTLE (3) bean embryos for short periods under sterile conditions. Isolated and immature embryos of species of *Rhapanus* and of *Cochlearia danica* have been grown with some success under sterile and non-sterile conditions by HANNIG (8). HABERLANDT (5) attempted to grow

[1] Published with the permission of the Director of the Agricultural Experiment Station, University of Missouri.

the isolated cells of the leaves of higher plants, and succeeded (**6, 7**) in obtaining some cell division under certain conditions in isolated pieces of the tissue from tubers, stems, and leaves of higher plants. To the writer's knowledge, however, the cultivation in sterile and artificial media of a portion of the meristematic tissue of higher plants has not been accomplished.

In the present paper a method is described by which isolated meristematic tissue (root tips and stem tips) of higher plants may be grown with some success under sterile controlled conditions. There are presented also the results of some preliminary experiments in which the method has been used, and which indicate the possibilities and limitations in growing excised root tips and stem tips under these conditions.

The experiments described were performed for the most part in 1917 at the Alabama Polytechnic Institute and Experiment Station, as the first step in an investigation to define the classes of materials required by a plant shoot or root for continued growth. In order to eliminate the influence of the shoot and its products on the root, or of the root on the shoot, it was considered necessary to grow the root tips isolated from the tops, and the shoot tips isolated from the roots, in artificial media under sterile conditions. It was thought that if the isolated meristematic tissue of a higher plant (such as the shoot tip or the root tip) could be cultivated successfully, such questions as the synthesis of elaborated nitrogen by the non-green parts of higher plants and the relation of chlorophyll and light to it, the necessity of accessory food substances for the growth of green plants, in short, the complete nutrient requirements of the shoot and of the roots of higher plants, and possibly of individual cells of the plant, could be investigated directly.

Method

The method followed in securing sterile root or shoot tips and cultivating the excised tissue was as follows. Seeds were sterilized by WILSON'S (**14**) calcium hypochlorite method, and transferred without washing to sterile Petri dishes containing a thin layer of 0.8 or 1 per cent plain agar. In transferring, a metal spoon made of aluminum with a wooden handle was used, and this spoon was

dipped in alcohol and flamed in order to sterilize it previous to use. When the seeds had germinated and the roots had reached a length of a centimeter or more, a centimeter or thereabouts of the root tip was cut off in the dish with a sterile scalpel, measured, and transferred with a sterile platinum loop to the described culture medium. The growing tip of the shoot was similarly treated. By this method a part of the plant, including the meristematic region, was excised and placed under sterile controlled conditions.

Growth of root tips in sterile nutrient solutions

Using the method described, the root tips of peas, cotton, and corn were placed in (1) a modified Pfeffer's solution,[2] (2) the same solution containing 2 per cent glucose, and (3) the modified Pfeffer's solution containing 2 per cent levulose. Each root tip was transferred to an Erlenmeyer flask of 125 cc. capacity, containing 50 cc. of solution. The flask was set in the dark and allowed to stand at room temperature during the period of the experiment. It was found that in such solution cultures the root tips of peas, cotton, and corn would develop into a considerable root system in the mineral nutrient solution containing carbohydrates, but that little growth occurred in the culture solution to which no carbohydrate was added. For all three plants the greatest growth occurred in the glucose solution. The method of culture and the appearance of root tips of corn in a 2 per cent glucose solution and in a solution lacking carbohydrates at the end of twenty-four days is shown in fig. 1. The detailed results of these early experiments were as follows:

PEAS.—In the experiment with peas, the variety Extra Early was used, and the period of growth was twenty-nine days. Fifteen root tips were used with each sugar solution and thirteen in the mineral solution without sugar. One contamination developed. All of the roots in the levulose solution were darkish brown, particularly at the cut end. Those in Pfeffer's solution were pure white. All were turgid, but the specific gravity of those in the Pfeffer's solution was less than that of those grown in the sugar solution.

[2] The composition of this solution was as follows:
$Ca(NO_3)_2$, 2 gm.; KH_2PO_4, 0.5 gm.; KNO_3, 0.5 gm.; KCl, 0.25 gm.; $MgSO_4$, 0.5 gm.; $FeCl_3$, 0.005 gm.; distilled water, 6000 cc.

The former floated on water, the latter sank. The average growth in length of the roots is given in table I, from which it can be noted

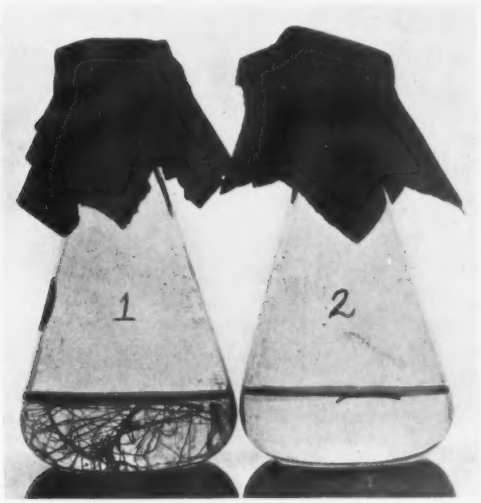


FIG. 1.—Appearance of root tips of corn in Pfeffer's solution at end of twenty-four days; flask no. 1 contains 2 per cent glucose, flask no. 2 contains no carbohydrates; root tip in no. 1 appeared as root tip does in no. 2 at beginning of experiment.

that in the mineral nutrient solution alone the average gain in length was 0.84 cm., and no secondary roots were produced. In levulose the average gain was 2.04 cm. with 2.3 secondary roots;

TABLE I

ROOT TIPS OF PEA GROWN TWENTY-NINE DAYS IN DARK

Solution	Number root tips used	Average original length (cm.)	Gain in length 29 days (cm.)	Side roots, average number per root
Pfeffer's solution.....	13	1.58	0.84	0
Pfeffer's plus glucose.....	14	1.49	4.27	3.5
Pfeffer's plus levulose.....	15	1.54	2.04	2.3

in glucose the average gain was 4.27 cm. with 3.5 secondary roots. Considerable variation in growth was evident. Some of the roots

in the sugar solution made little or no growth, others grew well (fig. 2). These roots which showed little growth lowered the average for the lengths in the sugar solutions. The maximum growth in glucose of a root tip originally 1.5 cm. long was 13.5 cm. with

FIG. 2.—(1) Root tips of pea grown in dark in Pfeffer's solution plus 2 per cent glucose; (2) plus 2 per cent levulose; (3) plus no carbohydrate; (4) corn root tips grown in Pfeffer's solution plus 2 per cent levulose; (5) plus no carbohydrate; (6) plus 2 per cent glucose.

thirteen side roots, in levulose of a root tip originally 2.6 cm. the maximum growth was 7.5 cm. with nine secondary roots.

The general appearance of the pea roots was not entirely normal. The brownish color, especially noticeable in the levulose solutions, and the failure of some of the roots in the sugar solution to make much growth while others grew fairly well, suggest that the sterili-

zation of the seeds, the method of handling the roots, or the culture solution was injurious or at least unfavorable. It is of interest to note that when the root curved in its growth the lateral roots were produced on the convex side of the root. This was found to be true also in the roots of all three kinds of plants, and it can be noted in the case of corn and pea roots in fig. 2. JOST (10) states that by bending the main root, development of lateral roots may be prevented from the concave side, and cites the lupine as an example after NOLL.

CORN.—Using the same methods, corn roots were investigated. The period of growth was eleven days in the dark, and the number of roots used in this preliminary experiment was three in the glucose solution, two in the levulose solution, and two in the mineral nutrient solution without sugar. The corn root tips made a much greater growth than the peas, and did not show the browning so evident in the pea roots in the sugar solution, but were white in both the sugar solution and the mineral nutrient solution at the end of the experiment. The data in table II show that the average

TABLE II

ROOT TIPS OF CORN GROWN ELEVEN DAYS IN DARK

Solution	Number roots used	Average original length (cm.)	Gain in length 11 days (cm.)	Average number secondary roots
Pfeffer's plus 2% glucose...	3	2.3	8.33	22
Pfeffer's plus 2 % levulose..	2	3.75	5.95	20
Pfeffer's solution.....	2	4.15	1.60	0

increase in length in the Pfeffer's solution was 1.6 cm. with no secondary roots, the average increase in length in the levulose was 5.95 cm. with twenty secondary roots, and in glucose 8.33 cm. with twenty-two secondary roots. The appearance of the corn roots at the end of the experiment is shown in fig. 2.

This preliminary experiment does not indicate, however, the maximum amount of growth which corn roots may make under the conditions described. Since the experiment was performed, several hundred corn roots have been grown in sterile nutrient solutions. Corn roots with a length of 14–17 cm. and with 80–125 secondary

roots have frequently been grown in two weeks in the dark in Pfeffer's solution containing 2 per cent glucose. The maximum length has been that of a tip originally 2.0 cm. long which attained a length of 32.5 cm. and 131 secondary roots, in forty-three days.

COTTON.—Root tips of cotton were grown seventeen days in the three solutions, and with them even more striking results were secured than with the corn. Tips of ten roots were used in glucose, ten in levulose, and eleven in Pfeffer's solution without sugar. The root tips in glucose lengthened very rapidly, and at the end of seventeen days, as indicated in table III, had attained an average

TABLE III

GROWTH OF ROOT TIPS OF COTTON IN STERILE NUTRIENT SOLUTIONS

Solution	Number of roots	Average original length (cm.)	Gain in length 17 days (cm.)	Average number secondary roots per root	Dry weight per root (gm.)
Pfeffer's plus 2% glucose.....	10	3.06	31.76	61	1224
Pfeffer's plus 2% levulose.....	10	3.11	2.34	29	0.0728
Pfeffer's solution.....	11	2.99	1.92	0.1	0.1240

length of 34.8 cm., while originally they were but 3.06 cm. long. The cut end of the roots in glucose was very dark brown, almost black for 1–1.5 cm., and brownish for 3–4 cm. The root cap was black. In levulose the increase was much less, only 2.34 cm., and the whole root was dark brown. The increase in length in the mineral solution alone was still less, being only 1.9 cm. The roots were pure white. The maximum growth in the glucose solution was that of a root tip 3.2 cm. long which attained a length of 42.2 cm. with seventy secondary roots. In levulose the maximum growth was that of a root tip 2.1 cm. long which grew to 13.4 cm. with thirteen secondary roots.

Growth of root tips in agar

The excised root tips of corn have also been grown in 1 per cent agar. Fig. 3 shows the growth of an excised root tip of corn at the end of two weeks in Pfeffer's solution containing 1 per cent

agar, and in Pfeffer's solution plus 2 per cent glucose containing 1 per cent agar. It can be noted from the figure that the root tip not supplied with glucose has made little growth, while the root tip supplied with glucose has made considerable growth, has responded normally to gravity, and has produced a considerable number of secondary roots.

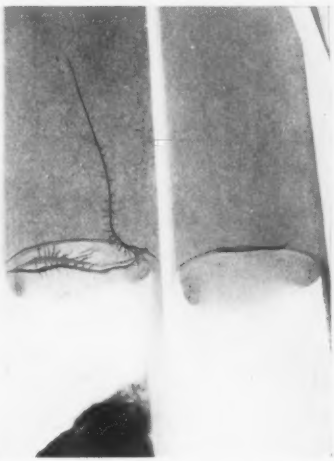


FIG. 3.—Growth of root tips in Pfeffer's solution plus 1.0 per cent agar; tube on left no glucose, tube on right 2 per cent glucose.

Growth of shoot tips in sterile nutrient solutions

The shoot tips of pea, corn, and cotton were placed in the three solutions and grown in the dark. While growth was secured in the carbohydrate solution with cotton, the development was abnormal and measurements were not made. The shoots of peas and corn developed more normally in the carbohydrate solution, and in many cases produced roots. The plants, however, were chlorotic and showed the elongation and small leaf development typical of plants grown in the dark (fig. 4). In both cases, as indicated in table IV, the greatest increase in length occurred in the glucose solution and least in the Pfeffer's solution without sugar. The relative growth of the shoot tips in the three solutions was therefore the same as that of the root tips. Starch was found in the tissue grown in the glucose and levulose solutions, and in the guard cells of the shoots in the Pfeffer's solution.

From these experiments it is evident that in the dark, in solution cultures containing the mineral salts commonly accepted as essential for the growth of green plants and a soluble carbohydrate, the root tips of corn, cotton, and peas, and the stem tips of corn, cotton, and peas make considerable growth. In the same solutions lacking

carbohydrates, only a slight increase in length occurs, probably at the expense of carbohydrate originally present in the root tip.

Glucose is apparently a better source of carbon than levulose for all three plants and for both tops and roots. This is particularly

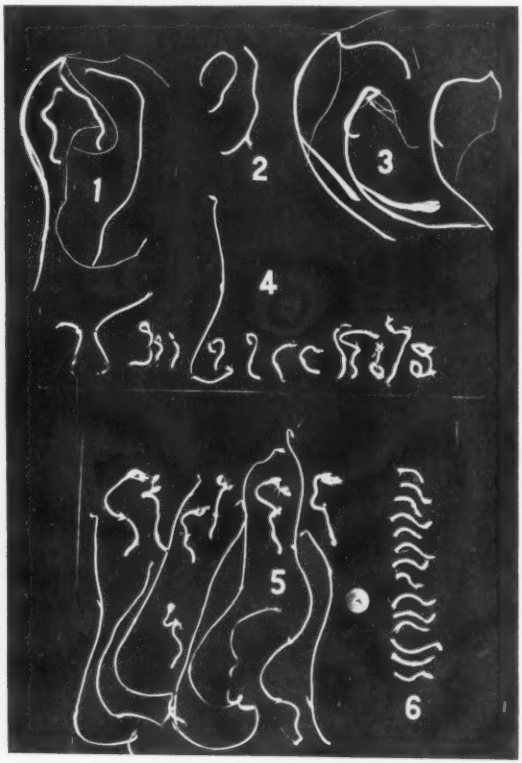


FIG. 4.—(1) Corn shoot tips grown in dark in Pfeffer's solution plus 2 per cent levulose; (2) plus no carbohydrate; (3) plus 2 per cent glucose; (4) pea shoot tips grown in dark in Pfeffer's solution plus 2 per cent levulose; (5) plus 2 per cent glucose; (6) plus no carbohydrate.

noteworthy in the case of cotton, where the increase in length of the root tips in glucose, as indicated in table III, was thirteen times the gain in levulose, twice as many secondary roots were produced,

and the gain in dry weight compared with the check was twice as great. Although the number of corn root and stem tips used in this experiment was small, the roots grown in glucose were better than those in levulose. No comparison between the two sugars could be made in the case of cotton stem tips. The contrast between the relative effect of glucose and levulose on the amount of growth made by the tissue of these seed plants and their effect upon the

TABLE IV

GROWTH OF SHOOT TIPS OF PEA AND CORN IN DARK, TWENTY-NINE AND ELEVEN DAYS RESPECTIVELY, IN STERILE NUTRIENT SOLUTIONS

Additions to modified Pfeffer's solution	Number shoot tips used	Average original length (cm.)	Gain in length (cm.)	Total number roots
	Peas			
Glucose (2%)	15	1.75	12.77	7
Levulose (2%)	15	1.72	3.58	3
None	14	1.75	0.88	14
	Corn			
Glucose (2%)	3	2.16	17.5	13
Levulose (2%)	3	2.90	12.43	8
None	2	3.75	4.50	1

growth made by *Ceratodon purpureus* should be noted. In the case of the latter plant, as reported earlier (**13**), the amount of dry matter produced with levulose as the carbon source was 2–7 times as great as the amount produced with glucose. The fact that the stem tips in the dark, even in the presence of 2 per cent glucose, are morphologically like etiolated shoots, would also indicate that it is not an absence of available carbohydrate which causes the stem elongation and small leaf development of plants grown in the dark.

Continued growth of root tips in culture solutions

The fact that the root tips in these experiments grew in solutions containing carbohydrates, and made very little growth in the same solutions lacking sugar, would suggest that the complete requirements for the growth of roots are water, mineral salts, carbohydrates, and free oxygen. If such were the case we should expect

that, furnished with sufficient quantities of these materials in the proper proportions, a root would continue to grow indefinitely. The excised roots of corn, however, will not grow indefinitely under the conditions described. Their development is rather definitely limited in the dark in the culture solutions used, as can be noted from the following experiment.

Root tips of corn were grown in the dark in the Pfeffer's solution containing 2 per cent glucose. At the end of eight days the tips of the roots were cut off and transferred to a fresh solution of the same composition, where they were allowed to grow for ten days. At the end of that time the tip was again cut off and transferred to a new solution. Growth took place there for ten days. If the nutrient solution were complete and no inhibiting or injurious factors active, by continued transfers we should be able to keep a root tip growing indefinitely in the same way as cultures of bacteria, molds, or yeasts are kept growing indefinitely by continued transfers to fresh media.

In this experiment the growth during the first period was excellent, and the number of secondary roots large (table V). During

TABLE V

GROWTH OF ROOT TIPS OF CORN IN NUTRIENT SOLUTIONS: ROOT TIPS IN GLUCOSE SOLUTION CUT OFF AND TRANSFERRED AS INDICATED

Period of growth	Number roots used	Average original length (cm.)	Gain in length (cm.)	Number secondary roots per root
	Pfeffer's solution plus 2 per cent glucose			
June 30–July 7	16	3.43	8.98	64.8
July 7–July 17	16	3.48	2.04	20.8
July 17–July 28	13	1.86	0.14	00.0
	Pfeffer's solution			
June 30–July 28	10	3.65	0.90	00.0

the second period the increase in length was one-fourth that of the first period, and the number of secondary roots one-third. In the third period hardly any increase in length was found, and no secondary roots were produced.

The maximum gain in the first period was that of a root tip originally 4.3 cm., which increased to 18.0 cm. and produced ninety-seven secondary roots. The maximum gain in the second period was that of a root tip 2.7 cm. long originally, which increased to 7.4 cm. and produced twenty-one secondary roots. In the third period only three out of thirteen roots showed any increase in length, the maximum being 0.9 cm. This experiment has been repeated many times, and many root tips of corn have been carried through the three periods of culture given. Five varieties of corn have been used: a dent variety from Alabama; Longfellow flint from New York (kindly furnished by Dr. J. K. WILSON); Boone County White and Reed's Yellow dent from Missouri; and Funk's Yellow dent from Illinois. The stoppage of growth in the third period in Pfeffer's solution plus 2 per cent glucose has occurred in every case. No single root tip thus far has made more than a slight amount of growth in the dark in the third period.

Not only does a diminution of growth rate, and a reduction in the production of secondary roots ending in a cessation of growth in the third period take place in the course of these transfers, but the diameter of the root tip continually decreases, until in the third period its diameter is one-fourth or less that of the original root tip. When stoppage of growth takes place, however, the root tips may apparently be normal in macroscopic and microscopic appearance, showing vascular bundles and root hair development.

The failure of an excised root to continue growth when repeated transfers of the root tip are made, at once suggests that the seedling root contains some material derived from the seed other than glucose, the mineral salts of Pfeffer's solution, water, and free oxygen which are necessary for continued growth and which the root cannot synthesize in the dark in solution cultures from the material supplied. Such material or materials would be fractionated by the continued transfers of the root tips.

Other explanations may be suggested. The stoppage of growth may be due to an unbalanced condition of the nutrient solution in which the roots are grown. The root tips, however, at the time of growth stoppage may show no macroscopic evidence of injury, and the fact that root hairs may be present on the root

tip in the third period also would indicate that the stoppage of growth is not due to the toxicity of the solution. The dextrose may penetrate too slowly to furnish sufficient carbohydrate to the root cells for continued growth. This would not appear to be a feasible explanation, however, because the early growth of the root tip is rapid, and the decrease in growth appears progressively greater. The early growth must occur at the expense of glucose which penetrates the root cells, because the root tips furnished with no glucose in the nutrient solution grew very little. It would seem that if the rate of penetration of the glucose determines the stoppage of growth one would have to assume a continuously increasing difficulty of penetration, terminating in entire impermeability of the root cells to glucose. The limited oxygen supply in the solution cultures may account for the growth stoppage. Here again, however, no better supply of oxygen is supplied in the first period than in the later periods, and yet the growth in the first period is the most rapid. If the limited oxygen supply is the factor which eventually causes the stoppage of growth, it must be a cumulative effect, due either to the development of deleterious materials or failure to synthesize some necessary material. It should also be noted that, although the aeration of water cultures of entire corn plants favors root and top development (ANDREWS and BEALS 1), the roots of an entire plant in unaerated water cultures do not show the stoppage of growth evident with the excised roots in solution cultures. It would seem reasonable to assume, therefore, as a working hypothesis from the experiments described, that oxygen, the mineral salts of Pfeffer's solution, glucose, and water are insufficient for the continued growth of excised corn roots.

Summary

1. A simple method of growing the isolated meristematic tissue of higher plants, excised root tips and stem tips, under sterile conditions is described.

2. The excised root tips of peas, corn, and cotton make considerable growth in the dark in solution cultures containing mineral salts and glucose or levulose.

3. The excised root tips of peas, corn, and cotton make little growth in the dark in solution cultures containing mineral salts and lacking carbohydrate.

4. The growth of the isolated root tips of peas, corn, and cotton is markedly greater in solution cultures containing glucose than in those containing levulose.

5. The excised roots of corn respond normally to gravity when grown on agar containing mineral salts and glucose.

6. The isolated shoot tips of peas and corn make considerable growth in the dark in sterile solution cultures containing mineral salts and glucose or levulose, but little in the absence of carbohydrates.

7. The excised shoot tips of corn and peas grown in sugar solutions remain chlorotic, and those of peas show the stem elongation and small leaf development characteristic of plants grown in the dark.

8. When the excised root tips of corn are grown for ten days or two weeks in the dark in a solution culture containing glucose and mineral salts, and the tip is then cut off and transferred to a fresh solution of the same type, the amount of growth in the second period is less than that in the first, and ceases in the third period.

UNIVERSITY OF MISSOURI
COLUMBIA, MO.

LITERATURE CITED

1. ANDREWS, F. M., and BEALS, C. C., The effect of soaking in water and aeration on the growth of *Zea Mays*. Bull. Torr. Bot. Club **46**:91–100. 1919.
2. ANDRONESCU, DEMETRIUS ION, Germination and further development of embryo of *Zea Mays* separated from the endosperm. Amer. Jour. Bot. **6**:443–452. 1919.
3. BUCKNER, G. D., and KASTLE, J. H., The growth of isolated embryos. Jour. Biol. Chem. **29**:209–213. 1917.
4. BRANNON, J. M., A simple method for growing plants. Amer. Jour. Bot. **8**:176–178. 1912.
5. HABERLANDT, G., Culturversuche mit isolierten Pflanzenzellen. Sitzungsb. Akad. Wiss. Wien. Math.-Natur Classe. **111B**:69–92. 1902.

6. HABERLANDT, G., Zur Physiologie der Zellteilung. Sitzungsb. König. Preuss. Akad. Wiss. Berlin. 318–345. 1913.
7. ———, Zur Physiologie der Zellteilung. Zweite Mitteilung. Sitzungsb. König. Preuss. Akad. Wiss. Berlin. 1095–1111. 1914.
8. HANNIG, E., Zur Physiologie pflanzliche Embryonen. Bot. Zeit. **62**:45–80. 1904.
9. HUTCHINSON, H. B., and MILLER, N. H. J., The direct assimilation of inorganic and organic forms of nitrogen by higher plants. Centralbl. Bakt. **30**^{2}:513–547. 1911.
10. JOST, LUDWIG, Lectures on plant physiology. Transl. by R. J. H. GIBSON. 1907.
11. KNUDSON, L., Influence of certain carbohydrates on green plants. Cornell Univ. Agric. Exp. Sta. Mem. **9**:1–75. 1916.
12. MAZÉ, P., and PERRIER, A., Recherches sur l'assimilation de quelques substances ternaires par les végétaux à chlorophylle. Ann. Inst. Pasteur. **18**:721–747. 1904.
13. ROBBINS, W. J., Direct assimilation of organic carbon by *Ceratodon purpureus*. BOT. GAZ. **65**:543–551. 1918.
14. WILSON, J. K., Calcium hypochlorite as a seed sterilizer. Amer. Jour. Bot. **2**:420–427. 1915.
15. ———, Device for growing large plants in sterile media. Phytopath. **10**:425–439. 1920.
16. URBAIN, A., Influence des matières de réserve de l'albumen de la grain sur le développement de l'embryon. Rev. Gen. Bot. **32**:125–130; 165–191. 1920.

INFLUENCE OF WHEAT SEEDLINGS UPON THE HYDROGEN ION CONCENTRATION OF NUTRIENT SOLUTIONS[1]

LINUS H. JONES AND JOHN W. SHIVE

Introduction

That the reaction of nutrient culture media bears a very important relation to their biological properties is a fact that is well recognized. Consideration of H ion concentration is of vital importance in connection with plant culture studies, not only because of the profound influence which this factor exerts upon the manner in which plants respond toward certain nutrient elements in the media, but also because of its intimate relation to plant growth in general.

The H ion concentrations of some nutrient solutions commonly used for plant physiological studies undergo rapid and pronounced changes in contact with the roots of growing plants, while the reaction of other nutrient solutions changes only slightly or not at all under similar conditions, owing to the fact that they possess strong buffer properties. The rate, direction, and degree of reaction change are dependent, of course, upon a number of different factors, some of the more important of which are the composition and concentration of the nutrient solutions, and the species, age, and activity of the plants. It is not the purpose of this paper, however, to consider the various factors involved in the relation of the plants to the reaction changes which they may be capable of bringing about in nutrient solutions in which they are grown, but to report briefly an experiment carried out for the purpose of comparing the various nutrient solutions, commonly used for plant cultures, with respect to the initial H ion concentrations of the solutions, and to study the reaction changes induced in them by contact with the roots of young wheat plants.

[1] Paper no. 68 of the Journal Series, New Jersey Agricultural Experiment Station, Department of Plant Physiology.

Procedure

Spring wheat of the Marquis variety was germinated on a net as described by SHIVE (15). Seedlings carefully selected for uniformity of size and vigor were transferred, when about 5 cm. tall, to SHIVE'S three-salt solution R_5C_2, after having been mounted in the double piece paraffined cork stoppers devised by TOTTINGHAM (18). The stoppers were of the proper size to fit quart fruit jars of colorless glass, which were used for culture vessels. Three seedlings were included in each culture. The cultures thus prepared were conducted during a time period of twenty-five days, with renewal of solutions every three or four days. When the seedlings were approximately thirty days old from the time of germination, cultures were selected in which the plants were about equal with respect to size and vigor in so far as this could be judged from careful observation. The plants of the selected cultures, without being removed from the cork stoppers in which they were mounted, were then taken from the three-salt solutions, the roots were washed by carefully dipping them several times into distilled water, allowed to drain, and were then placed in the nutrient solutions devised by the various authors. The glass jars to which the plants were transferred were like those from which they were removed. Each jar had a capacity of 900 cc.

The formulae of the nutrient solutions devised by the various authors are given in tables I and II in terms of gram-molecules per liter. The solutions made up according to the formulae in table I were corrected to a total osmotic concentration value of approximately 1.75 atmospheres by the method of the freezing point lowering, while those prepared according to the formulae in table II were similarly corrected to an approximate osmotic concentration value of 1.00 atmosphere. No iron was added to any of these solutions, except to those for which this element is mentioned in the formulae. The solutions devised by BIRNER and LUCANUS (1), CRONE (2), and SACHS (13) contained precipitates. In making the cryoscopic tests for the total osmotic concentration values of these solutions the precipitates were allowed to settle, after which samples of the supernatant solutions were drawn off by means of a pipette, the lowering of the freezing points determined, and the corrections made whenever necessary.

TABLE I

FORMULAE OF NUTRIENT SOLUTIONS COMMONLY USED FOR PLANT CULTURES, ALL WITH TOTAL OSMOTIC CONCENTRATION VALUE OF APPROXIMATELY 1.75 ATMOSPHERES AS DETERMINED BY METHOD OF FREEZING POINT LOWERING

Author of solution	Volume-molecular partial concentrations of salts employed											
	KH_2PO_4	$Ca_3(PO_4)_2$	$Ca(H_2PO_4)_2$	$Fe_3(PO_4)_2$	$Ca(NO_3)_2$	KNO_3	$NaNO_3$	$MgSO_4$	K_2SO_4	$CaSO_4$	KCl	NaCl
Birner and Lucanus (1)	0.0108	.....	.....	0.0043	0.0133	.....	.....	0.0061	.....	.....	.....	.....
Crone (2)	.....	0.0022	.....	0.0019	.....	0.0266	.....	0.0056	.....	0.0049	.....	.....
Detmer (3)	0.0035	.....	.....	.....	0.0149	.....	.....	0.0051	.....	.....	0.0083	.....
Hartwell, Wheeler, and Pember (5)	.....	.....	0.0027	.....	0.0136	.....	.....	0.0077	.....	.....	0.0075	.....
Knop (7)	0.0044	.....	.....	.....	0.0145	0.0059	.....	0.0050	.....	.....	.....	.....
Pfeffer (12)	0.0041	.....	.....	.....	0.0136	0.0056	.....	0.0046	.....	.....	0.0037	.....
Sachs (13)	.....	0.0025	.....	.....	.....	0.0155	.....	0.0065	.....	0.0075	.....	0.0076
Schimper (14)	0.0028	.....	.....	.....	0.0018	0.0048	.....	0.0040	.....	.....	.....	0.0083
Schreiner, Skinner (16)	.....	.....	0.0066	.....	.....	.....	0.0278	.....	0.0030	.....	.....	.....
Shive, R_5C_2 (15)	0.0180	.....	.....	.....	0.0052	.....	.....	0.0150	.....	.....	.....	.....
Tollens (17)	0.0038	.....	.....	.....	0.0128	0.0052	.....	0.0049	.....	.....	.....	0.0054
Tottingham, $T_3R_1C_4$ (18)	0.0108	.....	.....	.....	0.0101	0.0034	.....	0.0081	.....	.....	.....	.....

The initial H ion concentrations of the nutrient solutions were determined immediately before bringing the plant roots in contact with them. H ion measurements were then repeated for each solution throughout a time period of fifty-two hours, during which the solutions remained in contact with the roots of the growing wheat plants. Nine tests were made of each solution

TABLE II

FORMULAE OF SOME LIVINGSTON-TOTTINGHAM (9) THREE-SALT NUTRIENT SOLUTIONS (TYPES I–VI) AND OF TOTTINGHAM'S (18) SOLUTIONS $T_1R_4C_5$ AND $T_1R_3C_5$ MODIFIED (JONES AND SHIVE 6) BY SUBSTITUTING AMMONIUM SULPHATE FOR POTASSIUM NITRATE IN EQUIVALENT OSMOTIC CONCENTRATIONS; ALL SOLUTIONS HAD TOTAL OSMOTIC CONCENTRATION VALUE OF APPROXIMATELY 1.00 ATMOSPHERE

Type	Number	Volume-molecular partial concentrations								
		KH_2PO_4	$Ca(H_2PO_4)_2$	$Mg(H_2PO_4)_2$	$Ca(NO_3)_2$	$Mg(NO_3)_2$	KNO_3	$MgSO_4$	$CaSO_4$	K_2SO_4	$(NH_4)_2SO_4$
		Livingston-Tottingham three-salt solutions									
I.	R_3S_2	0.0072	.....	.....	0.0048	.....	.....	0.0072	.....	.....	.....
II.	R_5S_2	.....	.....	0.0019	0.0037	.....	.....	.....	.....	0.0094	.....
III.	R_4S_1	.....	0.0025	.....	.....	.....	0.0099	0.0074	.....	.....	.....
IV.	R_5S_1	.....	0.0019	.....	.....	0.0057	.....	.....	.....	0.0076	.....
V.	R_5S_4	.....	.....	0.0025	.....	.....	0.0074	.....	0.0098	.....	.....
VI.	R_4S_2	0.0093	.....	.....	.....	0.0047	.....	.....	0.0047	.....	.....
		Modified Tottingham solutions									
	$T_1R_4C_5$	0.0021	.....	.....	0.0073	.....	.....	0.0071	.....	.....	0.0014
	$T_1R_3C_5$	0.0021	.....	.....	0.0073	.....	.....	0.0024	.....	.....	0.0042

during this period of contact. The small quantities of solutions withdrawn from the culture jars for the purpose of making tests were not replaced, since only about 2 cc. of solution was required for each determination. The H ion concentrations were determined by the colorimetric method, using the double tube color standards described by GILLESPIE (4).

The formulae of the three-salt solutions given in table II were selected from series of the six type-solutions proposed by LIVINGSTON and TOTTINGHAM (9). One solution was selected from each of six series to represent the six different types.[2] The table also

[2] A detailed description of these six type solutions, together with directions for their preparation, may be found in a "plan for cooperative research on the salt requirements of representative agricultural plants prepared for a special committee of the Division of Biology and Agriculture of the National Research Council." Edited by B. E. LIVINGSTON, Baltimore. 1919.

contains the formulae of TOTTINGHAM's (18) solutions $T_1R_1C_5$ and $T_1R_3C_5$ modified (JONES and SHIVE 6) by substituting ammonium sulphate for the potassium nitrate in equivalent osmotic concentrations. It has previously been shown (6) that in the TOTTINGHAM solutions when thus modified, the direction of the reaction change induced by contact with the roots of young wheat plants is usually the exact opposite of that in the unmodified solutions. It was because of this fact, and also because these modified solutions are capable of producing excellent growth of young wheat plants, that they were included in the experiment.

Discussion

The initial P_H values of the nutrient solutions, the formulae of which are given in table I, and the P_H values determined at intervals during the period of fifty-two hours throughout which the solutions remained in contact with the plant roots, are given in table III. It will be observed from the data of this table that the

TABLE III

P_H VALUES OF NUTRIENT SOLUTIONS DETERMINED AT INTERVALS DURING CONTACT WITH ROOTS OF GROWING WHEAT PLANTS

AUTHOR OF SOLUTION	P_H VALUES									
	INITIAL	Duration of intervals in hours								
		2	4	6	8.5	24.5	27	30.5	33	52
Birner and Lucanus	4.3	4.5	4.6	4.7	4.8	5.0	5.0	5.2	5.2	5.5
Crone	6.6	6.7	6.6	6.5	6.5	6.5	6.5	6.5	6.5	6.6
Detmer	4.7	4.8	4.9	5.0	5.2	5.6	5.6	5.7	5.7	5.9
Hartwell, Wheeler, and Pember	4.0	4.0	4.1	4.5	4.4	4.7	4.7	5.0	5.3	5.7
Knop	4.6	4.7	4.8	5.0	5.1	5.3	5.3	5.4	5.5	5.7
Pfeffer	4.7	4.7	4.9	5.1	5.3	5.5	5.5	5.6	5.7	5.8
Sachs	6.7	6.8	6.6	6.5	6.5	6.5	6.5	6.5	6.5	6.6
Schimper	4.8	4.9	5.1	5.2	5.4	5.7	5.7	5.8	5.9	6.1
Schreiner and Skinner	4.2	4.2	4.4	4.4	4.6	5.4	5.5	5.7	5.9	6.1
Shive, R_5C_2	4.5	4.6	4.6	4.6	4.7	4.8	4.8	5.0	5.1	5.3
Tollens	4.6	4.7	4.9	5.1	5.1	5.5	5.5	5.6	5.9	5.9
Tottingham, $T_3R_1C_4$	4.6	4.7	4.8	4.9	5.0	5.2	5.2	5.3	5.4	5.5

initial P_H values of only two of these solutions are close to the neutral point. CRONE's (2) solution has an initial P_H value of 6.6, and SACHS's (13) solution has a corresponding initial value of 6.7.

The initial P_H values of all the other solutions range between 4.0 and 4.8.

The P_H values of CRONE'S and of SACHS'S solutions remained practically unaltered during the entire period of contact with the plant roots. This is probably what might be expected, since the initial P_H values of these solutions lie close to the neutral point, and since the maximum reaction change which the wheat plants are capable of producing in any of the solutions whose formulae appear in table I finally brings the H ion concentrations of these solutions very close to this point, either slightly below or slightly above a P_H value of 7.0, regardless of the initial H ion concentrations of the solutions.

The various solutions exhibit marked differences in the rates of reaction change in contact with the plant roots under similar experimental conditions. Of the solutions with initial P_H values below 5.0, SHIVE'S solution R_5C_2 exhibited the highest resistance to reaction change, while TOTTINGHAM'S solution $T_3R_1C_4$ showed only slightly lower buffer properties as indicated by resistance to reaction change produced by the plants during the fifty-two hour period of contact. On the same basis the solutions of SCHREINER and SKINNER (**16**), and of HARTWELL, WHEELER, and PEMBER (**5**) possess relatively low buffer properties. With respect to the solutions here considered, it appears in general that the resistance offered to reaction change resulting from contact with the roots of growing plants is dependent largely upon the volume-molecular proportions of the soluble phosphate salts contained in the solutions. Thus SHIVE'S solution R_5C_2, which contains the highest proportion of dihydrogen potassium phosphate, exhibited the highest buffer properties. This is in entire accord with the observations of MCCALL and HAAG (**10**), and of MEIER and HALSTEAD (**11**). In the present experiment, however, there is one striking exception to this general rule as exhibited by the solution of BIRNER and LUCANUS (**1**), which has a volume-molecular proportion of dihydrogen potassium phosphate equal to that in TOTTINGHAM'S solution $T_3R_1C_4$, and about two and one-half times higher than that in KNOP'S (**7**) solution or in PFEFFER'S (**12**) solution, yet these solutions showed a higher resistance to reaction change as influ-

enced by the growing wheat plants than did the solution of BIRNER and LUCANUS. These comparisons, of course, are made upon the assumption that the plants in all the cultures were approximately equal with respect to their ability to cause reaction change.

The P_H values recorded in table IV were determined in the same manner and at the same intervals during the same time period as were those given in table III. The initial H ion concentrations of the LIVINGSTON-TOTTINGHAM (9) solutions containing dihydrogen potassium phosphate are always much lower than

TABLE IV

P_H VALUES OF NUTRIENT SOLUTIONS DETERMINED AT INTERVALS DURING CONTACT WITH ROOTS OF GROWING WHEAT PLANTS

TYPE	NUMBER	P_H VALUES									
		INITIAL	Duration of intervals in hours								
			2	4	6	8.5	24.5	27	30.5	33	52
		Livingston-Tottingham three-salt solutions									
I.	R_3S_2	4.6	4.7	4.8	5.0	5.1	5.4	5.4	5.5	5.6	5.7
II.	R_5S_2	3.8	3.8	4.0	4.1	4.3	4.4	4.5	5.0	5.3	5.8
III.	R_4S_1	3.6	3.6	3.7	3.9	4.0	4.3	4.3	4.6	4.8	5.6
IV.	R_4S_1	3.7	3.8	3.9	4.0	4.1	4.3	4.3	4.5	4.7	5.6
V.	R_3S_1	3.6	3.6	3.7	3.8	3.9	4.3	4.3	4.3	4.4	5.4
VI.	R_4S_2	4.3	4.7	4.7	4.9	5.0	5.2	5.2	5.3	5.4	5.6
		Modified Tottingham solutions									
	$T_1R_1C_5$	4.8	4.9	4.9	5.0	5.0	4.7	4.6	4.6	4.6	4.2
	$T_1R_3C_3$	4.8	4.9	4.9	5.0	4.9	4.6	4.4	4.4	4.4	4.2

are those containing the corresponding calcium or magnesium salts. This fact is more certainly brought out by MCCALL and HAAG'S (10) table of P_H values determined for six complete series of the LIVINGSTON-TOTTINGHAM type-solutions described in a publication (8) prepared for a special committee of the Division of Biology and Agriculture of the National Research Council.

Mention should be made of the fact that the initial P_H values of the solutions here chosen as representatives of types III, IV, V, and VI, as given in table IV, are not in very close agreement with those determined for the same solutions by MCCALL and HAAG.

The initial P_H values of these solutions in the order given are 3.6, 3.7, 3.6, and 4.3, while McCALL and HAAG's values are 4.1, 4.1, 4.3, and 4.7 in the same order. Disagreements of this kind are to be expected, of course, because of the different methods employed in determining the P_H values and in the preparation of the solutions, variations in the degree of purity of the salts used, differences in the temperatures of the solutions at the time when the measurements are made, etc. A good example of such discrepancies appears in the P_H values of a series of the LIVINGSTON-TOTTINGHAM solutions of type I as determined by McCALL and HAAG and by MEIER and HALSTEAD. These authors do not agree upon a single solution of a complete series of twenty-one, the values determined by the latter authors always being considerably but uniformly higher than those determined by the former.

It will be observed from the data of table IV that the maximum reaction changes produced by the plants during the fifty-two hour period of contact with the LIVINGSTON-TOTTINGHAM solutions with low initial P_H values are always considerably greater than are the corresponding changes in the solutions with higher initial P_H values, since the final P_H values of all these solutions show no very marked differences. Experience with these type-solutions has shown that the maximum reaction changes which young wheat plants are capable of producing in them finally always brings the P_H values of the solutions very close to the neutral point, regardless of the initial P_H values, although the time required to accomplish this may vary considerably with the different solutions, owing to differences in their buffer properties.

As has previously been shown (**6**), the direction of the reaction changes of the modified TOTTINGHAM solutions during contact with the roots of the young wheat plants is usually the opposite of that of the LIVINGSTON-TOTTINGHAM solutions and of the unmodified TOTTINGHAM (**18**) solutions under similar conditions. It will be observed, however, that the maximum reaction changes produced in these solutions by the plants during the fifty-two hour period of contact are not very great, the change in the H ion concentration of both solutions here considered being from $P_H = 4.8$ to $P_H = 4.2$, although the total osmotic concentration value of these solutions is only 1.0 atmosphere.

Experience with twenty representative solutions of TOTTINGHAM's (18) complete series of eighty-four, modified as here described by substituting ammonium sulphate for the potassium nitrate in equivalent osmotic concentrations, has shown that the P_H values of these solutions are not greatly altered by contact with the roots of young wheat plants between the ages of four and five weeks, the tendency always being toward a slight increase in the H ion concentration of the solutions during growth intervals of three or four days without renewal of the solutions. It is thus easily seen that for certain types of culture studies in which it is desirable to maintain the H ion concentrations of the nutrient media within comparatively narrow variation limits, solutions of this kind possess marked advantages over those in which the H ion concentrations are rapidly decreased by the action of the plants. The two solutions (modified TOTTINGHAM solutions $T_1R_1C_5$ and $T_1R_3C_5$) have the added advantage of high efficiency in the production of young wheat plants when iron in small amounts is supplied to the solutions in an insoluble form such as ferric phosphate. Soluble iron in the form of ferrous sulphate, even in small traces, has been shown (6) to be exceedingly toxic to the plants grown in these solutions.

NEW JERSEY AGRICULTURAL EXPERIMENT STATION
NEW BRUNSWICK, N.J.

LITERATURE CITED

1. BIRNER, H., and LUCANUS, B., Wasserkulturversuche mit Hafer in der Agric.-Chem. Versuchsstation zu Regenwalde. Landw. Versuchsst. **8**:128–177. 1866.
2. CRONE, G., Ergebnisse von Untersuchungen über die Wirkung der Phosphorsäure auf die höhere Pflanzen und eine neue Nährlösung. Sitzungsber. Neiderrhein. Ges. Nat. und Heilk. Bonn. 1902 (pp. 167–173).
3. DETMER, W., Practical plant physiology, translated by S. A. MOOR. London. 1898 (p. 2).
4. GILLESPIE, L. J., Colorimetric determination of hydrogen-ion concentration without buffer mixtures, with especial reference to soils. Soil Science **9**:115–136. 1920.
5. HARTWELL, B. L., WHEELER, H. J., and PEMBER, F. R., The effect of the addition of sodium to deficient amounts of potassium upon the growth of plants in both water and sand cultures. Ann. Report Rhode Island Agric. Exp. Sta. **20**:299–357. 1907.

6. JONES, L. H., and SHIVE, J. W., The effect of ammonium sulphate upon plants in nutrient solutions supplied with ferric phosphate and ferrous sulphate as sources of iron. Jour. Agric. Res. **21**:701–728. 1921.
7. KNOP, W., Quantitative-analytische Arbeiten über den Ernährungsprocess der Pflanzen. II. Landw. Versuchsst. **4**:173–187. 1862.
8. LIVINGSTON, B. E., A plan for cooperative research on the salt requirements of representative agricultural plants prepared for a special committee of the Division of Biology and Agriculture of the National Research Council. Baltimore. 1919.
9. LIVINGSTON, B. E., and TOTTINGHAM, W. E., A new three-salt solution for plant cultures. Amer. Jour. Bot. **5**:337–346. 1918.
10. MCCALL, A. G., and HAAG, J. R., The hydrogen-ion concentration of certain three-salt solutions for plants. Soil Science **10**:481–485. 1920.
11. MEIER, H. F. A., and HALSTEAD, C. E., Hydrogen-ion concentration relations in a three-salt solution. Soil Science **11**:325–350. 1921.
12. PFEFFER, W., The physiology of plants, translated by A. J. EWART, **1**:420. Oxford. 1900.
13. SACHS, J., Vegetationsversuche mit Ausschluss des Bodens über die Nährstoffe und sonstigen Ernährungsbedingungen von Mais, Bohnen, und anderen Pflanzen. Landw. Versuchsst. **2**:219–268. 1860.
14. SCHIMPER, A. F. W., Zur Frage der Assimilation der Mineralsalze durch die grüne Pflanze. Flora **73**:207–261. 1890.
15. SHIVE, J. W., A study of physiological balance in nutrient media. Physiol. Res. **1**:327–397. 1915.
16. SCHREINER, O., and SKINNER, J. J., Ratio of phosphate, nitrate, and potassium on absorption and growth. BOT. GAZ. **50**:1–30. 1910.
17. TOLLENS, B., Über einige Erlichterungen bei der Kultur von Pflanzen in wässerigen Lösungen. Jour. Landw. **30**:537–540. 1882.
18. TOTTINGHAM, W. E., A quantitative chemical and physiological study of nutrient solutions for plant cultures. Physiol. Res. **1**:133–245. 1914.

SULPHUR AND NITROGEN CONTENT OF ALFALFA GROWN UNDER VARIOUS CONDITIONS

CONTRIBUTIONS FROM THE HULL BOTANICAL LABORATORY

E. H. HALL

Introduction

Various investigators show a considerable variation in the analyses of the sulphur content of alfalfa, as well as in the proportion of organic and inorganic sulphur in the crop. It was thought well to analyze alfalfa from a number of selected places to see to what degree these variations could be explained by different sources of the crop, and to what degree by the personal factor of applying the none too reliable fusion methods, which probably always involve more or less volatilization and loss of sulphur. The very extensive Oregon work (11) has shown that the acre yield of alfalfa is enormously increased on their plats by the application of any sulphur source, and that on the fertilized plats the alfalfa contains a very much higher percentage of protein. Determinations were made in each sample of the total and nitrate nitrogen in order to ascertain whether there is any correlation between the sulphur and nitrogen content of alfalfa from the various sources.

It has been long established that sulphur is one of the ten essential elements for the growth of plants. This is easy to understand when one recognizes that sulphur is an essential building material for all plant proteins, as well as for various odor and flavor producing organic compounds found in members of the mustard family, onions, etc. While much of the sulphur in plants is found in the form of these organic compounds, there is also some inorganic or sulphate sulphur present. The latter is generally considered an excess of absorption of sulphur over its utilization. It may be, however, that some free sulphate sulphur must be present as a building material in order to insure maximum protein synthesis and maximum growth. SCHERTZ found that in the older leaves of *Coleus Blumei* some free nitrate must be present as a building

material in order to prevent the decomposition of organic nitrogen compounds—proteins, chlorophyll, phospholipines, etc. A similar situation may hold in alfalfa for sulphate as a sulphur source.

On the basis of WOLFF's old ash analyses of crop materials, agricultural scientists came to assume that so little sulphur was used by crops that there was no doubt that all soils furnished an abundance of this material. The magnitude of error likely to appear in the ashing method is well illustrated by the fact that WOLFF's analyses showed that one hundred bushels of corn contain 0.2 lb. of sulphur, while analyses of the modern fusion methods show at least 8.5 lb. or 42.5 times as much (**2**). Ashing seems to drive practically all of the organic sulphur off into the air, and determines only the small amount of sulphur existing in the inorganic form.

The use of the fusion method (**7**) of determining the sulphur content of crops has quite changed the situation by showing that all crops are considerable users of sulphur, and some crops very heavy consumers of sulphur. This fact, together with the general low percentage of sulphur in soils and the large losses of sulphur from soils by leaching, has shown that the question of sulphur supply to crops needs serious consideration. The Oregon (**11**) and Washington (**9, 14**) stations have shown beyond doubt that alfalfa cannot be grown successfully on many lands of those states without the addition of a sulphur source. They commonly get increased tonnage amounting from 100 to 500 per cent by the use of gypsum or other sulphur sources. The protein content of the hay is also increased almost 2 per cent in some cases by the use of sulphur fertilizers. From these facts it seems probable that the marked benefits received from the use of land plaster on legumes and other high sulphur-using crops in eastern United States and England during the last 150 years are due to gypsum furnishing an excellent sulphur source (**3**).

HART and PETERSON (**5**) of Wisconsin, BROWN and KELLOGG (**2**) of Iowa, and SHEDD (**12**) of Kentucky have all emphasized the fact that a permanent fertility system must look after the sulphur supply of the soil as well as the so-called three fertilizer elements—nitrogen, potash, and phosphorus. Recent work is indicating that

the same is true of calcium, and some suggest that the same may sometimes be true for magnesium. It is not within the scope of this paper to give a full discussion of the present status of the sulphur fertilization problems in the United States. For a critical discussion of this problem the reader is referred to a paper recently written by CROCKER (3).

Methods

In order to study the chemical composition of hay produced in various localities, samples were secured from three different states, Kansas, Illinois, and Missouri. So far as it was possible, these samples were taken from several different places in the mow. This may not have constituted so good a representative sample as could have been secured from the field, but it was the best that could be obtained under the circumstances. The samples were dried for some time in the laboratory and then finely ground and the moisture determined. The samples aggregated about 100 gm. of the finely ground material. Aliquots of these samples were used for all the determinations. Table I gives a list of the samples, and, so far as possible, the types of soils and locality from which

TABLE I

Sample	Locality	Soil type	Crop
1.....	Circleville, Missouri	.....	Alfalfa
2.....	Meadville, Missouri	Grundy silt loam	Alfalfa
3.....	Brookfield, Missouri	Shelby loam	Sweet clover
4.....	Horton, Kansas	Silt loam	Alfalfa
5.....	Horton, Kansas	Brown silt loam	Alfalfa
6.....	Emporia, Kansas	Silt loam	Alfalfa
7.....	Emporia, Kansas	Silt loam	Alfalfa
8.....	Pratt, Kansas	.....	Alfalfa
9.....	Paris, Illinois	Brown silt loam	Alfalfa

they were taken. The following facts are necessary for a complete understanding of the table. The samples taken from Missouri were from farms that had never been under alfalfa cultivation until the last two years. The sweet clover had been harvested but once and was purely an experimental crop. The samples taken from Kansas were from some of the oldest and best alfalfa fields in the state. Some of these fields had produced maximum

crops for the past twelve years. Particular attention must be given to the samples from Paris, Illinois. Part of this field had been an old orchard where alfalfa had never been grown before. When the hay was ready to be harvested, parts of the field showed a difference in color, some being yellow and others being dark green. These samples when analyzed showed a marked chemical difference, as shown in the tables.

Several methods of sulphur determinations were tried before satisfactory results warranted the adoption of any particular one. The peroxide method as described in the Agricultural Chemists Bulletin no. 107 was finally adopted. The Osborn method caused a great deal of trouble by the igniting or foaming over of the material near the completion of the first fusion. This may have been due to too rapid heating of the crucible. This difficulty was better controlled in the official method because of the presence of sodium carbonate, which slowed down the reaction. The carbonate also caused some trouble at first in removing the residue from the crucible. This, however, was overcome by allowing a small stream of water to play on the residue after it had cooled just enough to prevent spattering. When the crucible was filled with water the material came out very easily. This treatment ruined the crucible in a very short time, but it shortened the process considerably.

There were two distinct crucial periods in the process. The first was when the sodium peroxide began to break down the material and ammonia was being liberated. This was the most critical because of the flashing which caused the loss of many determinations. Samples varied considerably in the flashing, perhaps owing to the different amounts of nitrate present. The second critical point occurred in the slow heating after the addition of the first 10 gm. of peroxide. The flame had to be regulated and the stirring so constant that the reaction did not become violent enough to burn up the sample or cause it to foam over the top. When these conditions were carefully controlled, the solutions were clear when neutralized, and the results obtained from a series of duplicate samples agreed as well as could be expected.

The dry fusion method is severely criticized by KOCH (STOCKHOLM 13) because not all the sulphur is secured by this method.

He states that in any dry fusion method there is some sulphur being lost in the fumes no matter how carefully the fusion is made. No experiments were made to test this statement, but the variation in results obtained from a number of analyses of the same sample leads one to believe that even with the official method some of the sulphur is not secure. It seems probable that in all determinations of the sulphur content of plant materials to date, the results are low because of the loss of sulphur in fusion. Some difficulty was experienced in controlling the fusion so as to prevent the reaction proceeding too rapidly. If the reaction becomes too violent, smoke is evolved in considerable quantities, and results obtained under these conditions are uniformly higher than those obtained when the fusion takes place at a moderate rate. At first the samples were considered lost although no flashing took place, but later these samples were saved, and in every case showed a higher value than those that were controlled perfectly, and agreed very closely in the final result. The rate of fusion may have a great deal to do with the amount secured from the sample. This point will be studied more extensively later. The evidence is that all the sulphur was not being obtained from the samples. KOCH advocates the use of perhydro, a very concentrated hydrogen peroxide, in the determination of sulphur, but unfortunately this substance has not been on the market since the war, as Germany was the sole manufacturer. OLSON (**8**) used the Parr bomb, and, when certain precautions are followed, claimed for it advantages in speed and in assurance against losses of sulphur. Modification of the method is reported in connection with more recent determinations (**9, 10**).

All the determinations were made on the air dry material, and the percentage of sulphur calculated to the oven dry weight as follows:

Number of grams in aliquot.....	1.25	1.25
Weight of $BaSO_4$ obtained.....	0.0328 gm.	0.0329 gm.
Percentage of sulphur in $BaSO_4$.....	13.73	.....
Percentage of moisture in sample....	5.82	5.82
Percentage of sulphur in sample.....	0.382	0.382

The BENEDICT method (**4**) was tried, but proved very unsatisfactory because of the extreme tendency of the material to sputter

at the time of fusion. This method is highly recommended by its author to give very close checks, even though they are lower than those of the peroxide method. BENEDICT attributes these high results in the peroxide method to silica, which is entirely lacking in his method. The writer experienced no trouble with silica in using the official method, for at no time was strong alkali allowed to stand in contact with glass without being neutralized. The ease with which the material was removed facilitated quick neutralization. There was never a weighable amount of silica found in the solution.

Sulphate sulphur determinations

The method of determining sulphate sulphur was the same as that used by AMES (1). Five grams of the dry material mixed with one per cent of hydrochloric acid were shaken in the mechanical shaker for three hours. The solution was filtered and an aliquot of 2.5 gm. taken. This was then treated with barium chloride and precipitation allowed to take place in the cold. After standing for at least forty-eight hours the sulphates were determined in the usual way. The averages of the determinations are given in table II.

TABLE II

Sample no.	Moisture	Weight $BaSO_4$	Total sulphur	Percentage total sulphur	Percentage sulphate sulphur	Total nitrogen	Nitrate nitrogen
	Circleville, Missouri (5 gm.)						
1	7.58	0.1304	0.0179	0.387	0.01	2.41	0.01
2	7.59	0.1320	0.0183	0.397	0.01	2.42	0.01
3	.....	0.1246	0.0182	0.371	.....	.....	.....
4	.....	0.1254	0.0171	0.373	.....	.....	.....
5	.....	0.1262	0.0173	0.375	.....	.....	.....
	Emporia, Kansas (1.25 gm.)						
1	5.81	0.0348	0.0047	0.405	0.013	2.32	.....
2	5.82	0.0369	0.0050	0.424	0.012	2.31	Trace
3	.....	0.0328	0.0045	0.382	.....	.....	.....
4	.....	0.0529	0.0045	0.383	.....	.....	.....
5	.....	0.0371	0.0051	0.432	.....	.....	.....

TABLE II—Continued

Sample no.	Moisture	Weight $BaSO_4$	Total sulphur	Percentage total sulphur	Percentage sulphate sulphur	Total nitrogen	Nitrate nitrogen
	Brookfield, Missouri						
1	8.57	0.0354	0.0048	0.425	0.024	2.09	0.01
2	8.43	0.0337	0.0045	0.390	0.027	1.97	0.01
3	.....	0.0319	0.0044	0.383	.....	.....	.....
	Horton, Kansas						
1	7.35	0.0430	0.0059	0.508	0.038	2.95	0.015
2	7.27	0.0476	0.0065	0.563	0.044	2.85	0.013
3	.....	0.0429	0.0059	0.507	.....	.....	.....
	Horton, Kansas						
1	8.45	0.0365	0.0051	0.440	0.02	2.66	0.01
2	8.54	0.0310	0.0041	0.374	0.027	2.71	.....
3	.....	0.0369	0.0051	0.440	.....	.....	.....
	Meadville, Missouri (1.25 gm.)						
1	8.81	0.0345	0.0047	0.408	0.02	2.39	Trace
2	8.83	0.0346	0.0048	0.409	0.024	2.36	.....
	Emporia, Kansas						
1	8.11	0.0345	0.0047	0.412	0.04	2.47	Trace
2	8.12	0.0346	0.0048	0.413	0.042	2.44	.....
	Pratt, Kansas						
1	5.82	0.0307	0.0042	0.358	0.012	2.38	Trace
2	5.88	0.0319	0.0044	0.370	0.011	2.36	.....
3	.....	0.0318	0.0044	0.370	.....	.....	.....
4	.....	0.0308	0.0042	0.358	.....	.....	.....
	Paris, Illinois (green alfalfa)						
1	7.08	0.0425	0.0058	0.503	.....	2.70	Trace
2	7.12	0.0414	0.0057	0.490	.....	2.86	.....
3	.....	0.0419	0.0058	0.495	.....	.....	.....
	Paris, Illinois (yellow alfalfa)						
1	5.81	0.0331	0.0045	0.386	.....	2.37	.....
2	5.94	0.0326	0.0045	0.380	.....	2.54	.....

Total nitrogen

Total nitrogen was determined by the ARNOLD-GUNNING method as modified to include nitrate nitrogen. This method is described by MATHEWS (6).

Nitrate nitrogen

The nitrate nitrogen was determined by the SCHLESING-WAGNER method as modified by KOCH. The method is described in detail by WOO (15) in his chemical study of *Amaranthus*. To test the accuracy of this method a five-tenths per cent solution of potassium nitrate was used. Theoretically, 2 cc. of this solution should give 2.22 cc. of gas calculated to standard conditions. The average result of several determinations was 2.15 cc., which was about 97 per cent of the calculated amount. The precaution necessary to insure the success of the process as described by WOO is that all the solutions must be entirely free from oxygen. The presence of oxygen tends to cut down the amount of gas absorbed, thus causing a low result.

In making the determinations, aliquots containing 5 gm. of the original samples were extracted with two 100 cc. of water for one-half hour each. This extract was then treated with lead acetate to precipitate the proteins which caused much trouble unless removed. The samples were made up to volume of 250 cc. and filtered, then 100 cc. of the clear solution representing 2 gm. of the dry material was concentrated on the steam bath to about 20 cc. and the NO gas determined. The following represents a determination as run in duplicate from a sample:

Aliquots in cc. (2 gm.)	100	
Total volume of gas evolved	1.66	cc.
Volume of unabsorbed gas	0.91	cc.
Volume of absorbed NO gas	0.75	cc.
Barometic pressure 749.7 mm., temperature	24.5	
Volume at standard conditions	0.68	cc.
Equivalent milligrams of KNO_3	0.0076	
Equivalent milligrams of NO_3	0.005	

Discussion

It is important to note the variation in the sulphur content of the hay from the widely separated districts. From the tabulated

results it will be seen that the amount of sulphur in the various hays analyzed runs from 7.4 to 11.16 lbs. per ton of hay. If an acre produces from 5 to 8 tons of hay annually, 37 to 90 lbs. of sulphur will be removed from each acre of soil each year. The maximum figures here are much higher than those of PETERSON, because of the very high sulphur content of the samples from Kansas. The analyses of the samples from Missouri and Illinois give results that agree more nearly with the results of PETERSON'S analyses of alfalfa from Wisconsin.

One other marked deviation from the results reported by PETERSON is seen in the low amount of sulphate sulphur obtained in these analyses. He found that the ratio of the organic to inorganic sulphur was practically unity. In table II the sulphate sulphur in no case exceeds 10 per cent of the total sulphur in the crop, and in the samples taken from Illinois there was no sulphate sulphur. On the average for all analyses the sulphate sulphur equals 4.35 per cent of the total sulphur. The following shows the percentage of the sulphur that existed in the inorganic form in the alfalfa collected from various regions:

	PER CENT
Paris, Illinois (1)	0.0
Paris, Illinois (2)	0.0
Circleville, Missouri	2.5
Emporia, Kansas (1)	3.0
Pratt, Kansas	3.2
Meadville, Missouri	5.3
Brookfield, Missouri (sweet clover)	6.2
Horton, Kansas	7.6
Emporia, Kansas (2)	9.9
Average	4.35

Summary

1. Alfalfa hay grown in various parts of the United States shows considerable difference in the percentage of total sulphur content, quite independent of sulphur fertilization.

2. In general, hay from fields with the heaviest crops contains the highest percentage of sulphur.

3. Good to excellent crops of alfalfa hay remove annually from thirty-seven to ninety pounds of sulphur per acre, an amount which would seem far in excess of the amount returned by rain.

4. In some samples all, and in every sample more than 90 per cent, of the total sulphur was in the organic form. There was none or little sulphur present in excess of the actual needs as building material.

This investigation was conducted under a research fellowship from the Gypsum Industries Association. The work was performed at the University of Chicago in the Hull Botanical Laboratory under the direction of Dr. WILLIAM CROCKER. The writer wishes to thank the Gypsum Industries Association for their kindness in furnishing the fellowship, and Dr. CROCKER for his advice and criticism. Thanks are also due Dr. FREDERICK KOCH for his advice and criticism of analytical methods.

STATE NORMAL SCHOOL
CHARLESTON, ILL.

LITERATURE CITED

1. AMES, J. W., and BOLTZ, G. T., Tobacco, influence of fertilizers on composition and quality. Ohio Agric. Exp. Sta. Bull. 285. pp. 173–209. 1915.
2. BROWN, P. E., and KELLOGG, E. F., Sulphur and permanent soil fertility in Iowa. Jour. Amer. Soc. Agron. 7:97–108. 1915.
3. CROCKER, WILLIAM, History of the use of gypsum as a fertilizer (unpublished).
4. HALVERSON, J. O., Estimation of sulphur in feeds and feces. Modified Benedict method for the estimation of sulphur in feeds, feces, and foods. Jour. Amer. Chem. Soc. 41:1494–1503. 1919.
5. HART, E. B., and PETERSON, W. H., Sulphur requirements of farm crops in relation to the soil and air supply. Wis. Agric. Exp. Sta. Bull. 14. pp. 21. 1911.
6. MATHEWS, A. P., Physiological chemistry. 2d ed. New York. 1916.
7. Official and provisional methods of analysis. Association of Official Agricultural Chemists. U.S. Dept. Agric. Bur. Chem. Bull. 107. pp. 272. 1912.
8. OLSON, G. A., The estimation of sulphur in plant material and soil. Wash. Agric. Exp. Sta. Bull. 145. pp. 12. 1917.
9. ———, Experiments with sulphur. Washington Agric. Exp. Sta. Annual Reports 1914, 1915, 1916, 1917, 1918, 1919, 1920.
10. OLSON, G. A., and ST. JOHN, J. L., Sulphur as plant food. Washington Agric. Exp. Sta. Bull. 165. pp. 69. 1921.
11. REIMER, F. C., and TARTAR, H. V., Sulphur as a fertilizer for alfalfa in Southern Oregon. Oregon Agric. Exp. Sta. Bull. 163. pp. 40. 1919.

12. SHEDD, O. M., The sulphur content of some typical Kentucky soils. Ky. Agric. Exp. Sta. Bull. 174. pp. 213–250. 1913.
13. STOCKHOLM, MABEL, A quantitative method for the determination of total sulphur in biological material. Master's thesis. University of Chicago. 1919.
14. THATCHER, R. W., and OLSON, G. A., Experiments in fertilizing alfalfa. Washington Agric. Exp. Sta. Popular Bull. 49. pp. 4. 1912.
15. WOO, M. L., Chemical constituents of *Amaranthus retroflexus*. BOT. GAZ. 68:313–344. 1919.

CURRENT LITERATURE

BOOK REVIEWS

Root systems

WEAVER[1] has made another notable contribution to our knowledge of root systems. Former investigations, noted in this journal,[2] included many root systems of grassland plants, and the present publication is confined to grassland vegetation and to the crop plants grown within its limits. The grasslands are considered under the three subdivisions of true prairie, mixed prairie, and short-grass plains.

The true prairie is characterized by tall sod-forming grasses growing in soil of rather abundant water content, with greater moisture in the subsoil. On the basis of root development, three general classes may be recognized in grassland vegetation. In the first the "working depth," or average depth reached by a large number of roots, is about 1.5 feet, with a maximum depth of 3.3 feet. The second class possesses roots with a working depth of 3.3 feet and a maximum of about 6 feet; while in the third class the working depth of the roots is usually 5–8 feet and the maximum penetration 8–12 feet, with a few species reaching an extreme of 15–20 feet. Examples of the three classes are *Aristida oligantha*, *Elymus canadensis*, and *Koeleria cristata;* *Andropogon scoparius*, *Bouteloua gracilis*, and *Grindelia squarrosa;* and *Andropogon furcatus*, *Aster multiflorus*, and *Panicum virgatum*. The deeper rooted species have few roots in the surface layers of the soil, showing a grouping of roots into more or less definite layers, thus reducing competition and permitting the growth of a larger number of species. In the short grass plains practically all plants have root systems well adapted for water absorption from surface soils. Two have roots with a working depth less than 2 feet, three have working depths of 2–4 feet, and three have a range of 4–7 feet. Examples of the three classes are *Opuntia polyacantha*, *Bulbilis dactyloides*, and *Psoralea tenuifolia*. Here the water supply is much more limited, especially in the subsoil. The soil and moisture conditions, as well as the vegetation in the mixed prairie, are intermediate between the true prairie and the short-grass plains. Compared with the true prairie, the plants are not as deeply rooted, but have usually developed a very efficient and widely spreading absorbing system in the surface soil.

The root systems of cereal crops grown at many stations in true and mixed prairie and short-grass plains were also examined. The comparative amount

[1] WEAVER, JOHN E., Root development in the grassland formation. Carn. Inst. Wash. Publ. 292. pp. 151. *pls. 23. figs. 37.* 1920.

[2] BOT. GAZ. **69**:351–353. 1920.

of root development of cereals in each seems to be in true prairie 100 per cent, in mixed prairie 80–95 per cent, and in short-grass plains 51–79 per cent. The experimental data are given in the form of tables, drawings, and photographs, all of excellent quality. It is recognized that variations in root development are caused by various factors, such as the chemical and physical character of soils and the evaporating power of the air. The soil factors are most effective through water content and aeration. The water relations of the various habitats were examined by WEAVER by means of atmometers and by soil moisture determinations, the latter being interpreted by means of the wilting coefficient and hygroscopic coefficient. He calls attention to the recognized fact that many plants are able to continue to absorb water below the limits of the wilting coefficient. In fact, it is in the responses of different plants to the use of soil moisture lying between the limits of the wilting and the hygroscopic coefficients that differences appear which might be used with advantage for a most significant classification. All wilting, whether clearly manifest or not, takes place at about the same moisture content, that is, at the wilting coefficient. As the moisture passes below this point the hydromesophytes soon die, the mesophytes live for a somewhat longer time, while xerophytes or drought-resistant plants prolong their existence for a very considerable period, reducing the soil moisture to the hygroscopic coefficient. It seems rather clear from the work of ALWAY and others, however, that the water absorbed below the limits of the wilting coefficient is quite insufficient for growth and merely serves to sustain life, indicating that the term "growth water" has been correctly used by the reviewer and others for the amount of soil moisture in excess of that indicated by the wilting coefficient.

While all of WEAVER'S investigations of root development have been of the highest order, this report shows a decided advance, for the accumulation of data has become sufficient to permit some significant generalizations. Among other things he points out that as our knowledge of root development in various associations increases it will render more accurate our interpretation of the indicator significance of the natural vegetation. Thus the contribution he has made to the science of ecology becomes most useful in the practice of agriculture.—GEO. D. FULLER.

Sturtevant's notes on edible plants

The purpose of this large volume,[3] as indicated in the preface, is that new knowledge may be available as follows: (1) the original home of many esculents is given for the first time; (2) new landmarks in the histories of edible plants are pointed out; (3) an effort is made to mention all cultivated esculents; (4) although the book contains much new information as to the history of the

[3] HEDRICK, U. P. (editor), STURTEVANT'S notes on edible plants. Report N.Y. Agric. Exper. Sta. for 1919. 2:4to. pp. vii+686. Albany: J. B. Lyon Co., State printers. 1919. $2.75.

food plants of the Old World, it is especially full and accurate in the discussion of the esculents of the New World; (5) STURTEVANT presents much new information on the variations that have been produced in plants by cultivation; (6) the book adds much to geographical botany; (7) many data are contributed toward the study of acclimatization.

The material contained in the book was compiled from the notes and manuscripts of the late Dr. E. LEWIS STURTEVANT, first Director of the New York Agricultural Experimental Station, and represents the labors of at least a quarter of a century. The editor and his assistants have had no light task in selecting from the large mass of partially organized material, and in verifying and organizing the bibliography, which includes some 6000 citations contained in 500 publications. This task of organization seems to have been well done. The information is arrayed in encyclopedic entries under the scientific name of the plant. To further facilitate reference there is an index of common names. The articles vary in length from a few lines for many of the less important plants to six or eight pages for such plants as beet, potato, tomato, and strawberry, and twelve pages for squash, pumpkin, and corn.

A portrait of STURTEVANT, a biographical sketch, and a bibliography of his writings add to the interest of the volume. Its comprehensive nature, including reference to some 3000 plants, its close attention to historical and geographic data, and its numerous citations of literature make it invaluable for reference. It greatly expands and often corrects details of the knowledge formerly available through such works as DE CANDOLLE'S *Origin of cultivated plants*. It is excellently printed, remarkably low priced, and is available for purchase from the New York Agricultural Experiment Station, Geneva, N.Y.—GEO. D. FULLER.

Devonian floras

ARBER'S[4] posthumous volume, which was sent to the press by the deceased author's wife and his friend D. H. SCOTT, gives a very welcome general survey of the Devonian floras. There is comparatively little known of the Devonian plants, and a great deal of what was formerly attributed to Devonian turned out to be of a later geologic age. On the other hand, the great discoveries of lower Devonian plants at Rhynie in Scotland, which represent the oldest known type of land plants, had given a renewed interest to Devonian paleobotany.

ARBER divides the fossil plants of the Devonian formation into two floras. The first and older division, which he calls *Psilophyton* flora, is represented by those low pteridophytes of which some types had already been described by Sir WILLIAM DAWSON in 1859, and whose affinities have remained so doubtful. The latest addition to this group is the newly discovered plants from Rhynie.

[4] ARBER, E. A. NEWELL, Devonian floras, a study of the origin of Cormophyta. 8vo. pp. xiv+100. *figs.* 47. Cambridge. 1921.

The second flora characterizes the middle and upper Devonian, and is called by ARBER *Archaeopteris* flora, because this fern predominates.

Other very interesting early types occurred in the upper Devonian, like *Archaeosigillaria*, *Protolepidodendron*, and numerous other earlier representatives of *Sphenophyllum*, *Sphenopteris*, and *Pseudbornia*.

This fascinating and very instructive little volume, whose value is increased by an extensive bibliography on the subject, forms a suitable monument for ARBER, who has been removed from the world of science at too early an age.—A. C. NOÉ.

North American slime-moulds

In 1899 MACBRIDE'S *The North American slime-moulds* was published, which was the first comprehensive presentation of this interesting group for American botanists. A brief review of that work was published in this journal.[5] A second edition has just appeared,[6] the first edition "having been exhausted long ago." It corrects certain errata of the first edition, but chiefly it incorporates the results of research during recent years. The painstaking work and clear statement which characterize MACBRIDE have resulted in a book of unusual quality. It is intended especially for American students, and therefore discusses chiefly American species, but it also includes brief descriptions of other forms, and refers to many extra-limital species now generally recognized. To many botanists it will probably give a new perspective of a group of organisms often dismissed with too little attention.—J. M. C.

MINOR NOTICES

Manual of woody plants.—TRELEASE[7] has published a second, revised edition of his small pocket manual of the woody plants used for decorative purposes. The intention of the author "is to make it possible for any careful observer to learn the generic and usually the specific name of any hardy tree, shrub, or woody climber that he is likely to find cultivated in the United States." The need of a new issue indicates that the manual has found a constituency. In the revised edition certain errors have been corrected, and the scope of the book has been enlarged by including a few additional types.—J. M. C.

British forestry.—A handy little volume by HANSON[8] gives in a non-technical manner the general principles of forestry as practiced in the British Isles and adjacent parts of Europe. In addition to plain directions for nursery

---

[5] BOT. GAZ. **29**:74. 1900.

[6] MACBRIDE, THOMAS H., The North American slime-moulds. A descriptive list of all species of Myxomycetes hitherto reported from the continent of North America, with notes on some extra-limital species. 8vo. pp. xvii+299. *pls. 23*. 1922.

[7] TRELEASE, WILLIAM, Plant materials of decorative gardening: The woody plants. 2d ed. 16mo. pp. xliii+177. Urbana: Published by the author. 1921. $1.00.

[8] HANSON, C. O., Forestry for woodmen. 12mo. pp. 238. *pls. 13. figs. 15*. 2d ed. Oxford. 1921.

and forest management, sawing and planting, felling and measurement, there are chapters on forest animals, birds, insects, weeds, and fungi. The final chapter deals with the uses of British timber. The book is in an easily readable style and seems to give a good idea of forestry as practiced in Great Britain.—GEO. D. FULLER.

NOTES FOR STUDENTS

Vegetation and climate.—The most recent phase of ecology, which finds expression in attempts to introduce quantitative methods for the investigation of vegetation and of the factors that determine its nature and distribution, has received a notable contribution in a recent volume by LIVINGSTON and SHREVE.[9] "The existence, limits, and movements of plant communities are controlled by physical conditions" is stated as a fundamental law of plant geography, and an attempt is made to map the distribution both of various physical factors and of a considerable number of plants and plant communities. Data for the latter are obtained from a variety of sources, and more particularly from SHREVE's well known map of the vegetation areas of the United States. In fact a generalized form of this map is employed as a basis upon which to display the distribution of climatic factors. In addition to this, ranges of various plant species and groups of species, such as deciduous, microphyllous, and broad-leaved evergreen trees, are delimited. Here emphasis is placed upon the lack of anything like an adequate knowledge of the ecological distribution, based on relative abundance, dominance, or density of stand, of any considerable number of plants even in such a well explored land as our own.

A general discussion of the influence of the environment on plant life classifies environmental factors which are important in distribution as: (1) moisture conditions, (2) temperature conditions, (3) light conditions, (4) chemical conditions, (5) mechanical conditions. These are considered in turn. In discussing the supply of water to vegetation the "residual soil moisture content" of the soil is not regarded as a soil constant, in spite of many data tending to prove that within very considerable limits its constancy holds. No mention is made of wilting or hygroscopic coefficients, nor of the notable contributions of such well known investigators as ALWAY, SHULL, KEEN, and BOUYOUCOS. These omissions constitute the most serious defect in an otherwise admirable and comprehensive volume.

The tabulation of the climatological data, used for the construction of the maps, in a form that makes them available for future investigators is highly to be commended. As an example of such accumulations of exact information regarding conditions limiting growth and distribution, the table of frost data may be cited. Here for 1803 different stations the altitude, number of years of record, average date of the last frost in spring, and the earliest in the

[9] LIVINGSTON, B. E., and SHREVE, F., The distribution of vegetation in the United States as related to climatic conditions. Carn. Inst. Wash. Publ. 284; pp. xvi+590. *pls.* 73. *figs.* 74. 1921.

fall, together with the length of the average frostless season, are given in readily accessible form, the resulting tables covering thirty-three pages. These data are plotted as isoclimatic lines on the map of the United States. Among the factors thus tabulated and mapped are: temperature efficiencies for the frostless season expressed as (1) remainder indices, (2) expotential indices, and (3) physiological indices; absolute temperature maxima and minima; average daily temperature for the coldest and hottest weeks of the year; mean daily precipitation for the frostless season together with the number of rainy and dry days for same; normal annual precipitation; atmospheric evaporating power; ratios of precipitation to evaporation; aqueous vapor pressure; relative air humidity; wind velocity; sunlight; and moisture temperature indices.

It is recognized that there are decided difficulties in establishing correlations of these isoclimatic areas with the distribution of plant species, growth forms, and vegetational areas, but even here the efforts of the authors have met with considerable success. The statement of climatic extremes for various vegetational features, in 128 tables covering 86 pages, certainly gives more exact information than was ever before available regarding the conditions under which various plant communities and plant species have developed. A very decided addition to our knowledge of the exact conditions that probably determine general vegetational areas is also provided in the plotting of the comparative ranges and intensities of twelve leading climatic conditions for nine such areas, for the life-zones of Merriam, and for over thirty plant species.

The book shows the uniformly good printing of text and maps characteristic of the publications of the Carnegie Institution of Washington, and seems reasonably free from errors of typography and in the use of specific names. It will be indispensable to all ecologists who wish to take account of climatic factors, and will become increasingly useful as increasing knowledge permits more accurate interpretation of such factors and their closer correlations with the resulting displays of plant life.—GEO. D. FULLER.

Anatomy and biology of gymnosperm leaves.—While there have been several investigations of leaves in various groups of gymnosperms, there has been no comprehensive study of the entire line. Consequently, a recent work by FEUSTEL[10] will be welcomed by those who wish to find, in compact form, a survey of the literature of the subject. The author states frankly that his work is only a summary of the literature, not an investigation; but his observations, especially along biological lines, and the comparative presentation of anatomical features are suggestive. The various orders are treated separately.

CYCADOFILICALES.—The term Pteridospermae is used for this order. The mode of treatment is similar in the other orders. In general appearance the leaves are fernlike, but the internal structure shows a mixture of fern and

[10] FEUSTEL, HERM., Anatomie und biologie der Gymnospermenblätter. Beih. Bot. Centralbl. **38**:177–257. 1921.

cycad characters. He notes the multiple leaf traces of the Medullosae, the concentric bundles in the rachis of *Lyginodendron* and *Heterangium*, and in the leaf of *Sutcliffia;* and among the cycad characters, the double leaf trace of *Lyginodendron*, and the secretory canal system. The leaves belong to the leathery type of recent ferns. The thickness and inrolled margins are xeromorphic characters, and the prevalent hypodermal sclerenchyma is xerophytic.

CYCADALES.—The review of the literature of the cycad leaf is particularly thorough, probably because there have been two rather extensive investigations. In the various genera and species, the shapes of cells of the epidermis, the stomata, the parenchyma, the thick-walled cells, and the vascular system are treated under separate headings. The xerophytic features are emphasized. The leaf structure is so characteristic in the different genera that a taxonomic key, based upon leaves, is presented. Since no study has been made of the leaves of *Microcycas*, this genus is not mentioned. Doubtless most of the investigations have been made upon leaves taken from greenhouse specimens. While the general structure is probably about the same as in plants in their native habitats, we should expect to find the xerophytic characters more pronounced in plants exposed to the extreme xerophytic conditions than in greenhouse plants, which are more or less shaded and are frequently watered.

BENNETTITALES.—So little is known of the internal structure of the mature leaf that this section is very brief, but there is a mixture of fern and cycad characters, and, according to FEUSTEL, some angiosperm characters.

CORDAITALES.—This order is treated under the separate headings Poroxyleae, Pityeae, and Cordaiteae; but since no leaves are known in the Pityeae, the study deals only with the other two groups. Resemblances to some of the Cycadofilicales and to some of the recent cycads are pointed out, but it is very questionable whether the similarities are due to relationship. Resemblances between the leaves of *Cordaites* and some of the Coniferales, especially *Agathis*, seem more striking.

GINKGOALES.—The heterophyllous leaves of *Ginkgo* are significant, the lobed and divided character being retained from the ancient forms. The structure of the leaf, with its long petiole, broad blade, and soft consistency, is not very xerophytic, but indicates that *Ginkgo* in its phylogeny has come from a climate with long wet periods.

CONIFERALES.—The structure and biology of the leaf of *Pinus* are treated in great detail as a type of the order, and the other genera are considered from the standpoint of comparative morphology and biology. The leaves of all the conifers, by their form, structure, and consistency, are protected against wind and rain. They are both xeromorphic and xerophytic. Several ecological hypotheses are advanced to account for the geographical distribution of the group.

GNETALES.—Naturally, the genera of this group are treated separately; but, in spite of the striking differences, the three genera show more resemblances to each other than to the rest of the gymnosperms. The leaves of

Gnetum resemble those of angiosperms in their internal structure as well as in their general appearance.

The conclusion for the entire group of gymnosperms is that the leaves belong to a single xerophytic type, with *Ginkgo* and *Gnetum* as the only exceptions. The literature list is very incomplete, because it was not thought necessary to repeat references which can be found in standard texts.—C. J. CHAMBERLAIN.

Respiration of thermophiles.—The respiratory activity of the thermophile fungi, *Thermoascus aurantiacus*, *Anixia spadicea*, and others, has been studied by NOACK,[11] who finds that the high respiratory activity is directly related to the rapid growth rate of these organisms, and that it is merely a consequence of the high temperature, not due to specific constitution or peculiar enzyme equipment. The economic coefficient for young cultures is 1.8, and about 3.6 for older ones. The respiratory quotient with changing oxygen supply and different growth rates from changed sources of carbon remains near one, so that the only peculiarity is the high respiration. From a comparison of the temperature coefficient of respiration in thermophiles, which is about 1.7 within the temperature limits for growth (35°–55°), with that of *Penicillium*, which is about 2 at 15°–25° C., NOACK concludes that the thermophiles show a restricted respiration. Thus, *Thermoascus* produces 310 per cent of its dry weight of CO_2 in 24 hours. If the respiratory rate of *Penicillium* at 25° C. were quadrupled by a rise to 45° C., however, it would produce 532 per cent of its dry weight of CO_2 in 24 hours. From this consideration of the VAN'T HOFF rule, and the absence of abnormal behavior in respiration and growth, he concludes that the high respiration of thermophiles is merely a temperature consequence, and is really somewhat restricted for that temperature.

With regard to this use of the VAN'T HOFF rule, and the finding of a lower temperature coefficient of respiration for thermophile fungi at 45° C. than for *Penicillium* at 25° C., attention is called to a recent paper by MATISSE,[12] who criticizes the use of the VAN'T HOFF rule, and urges the adoption by biologists of the ARRHENIUS temperature law instead. The formula for the VAN'T HOFF rule is incompatible with that developed by ARRHENIUS, and the latter is now accepted universally by physical chemists. The curves developed from each formula are much alike at low temperatures, but the ARRHENIUS formula shows that as the temperature goes higher, the value of Q_{10} decreases. The lower temperature coefficient for thermophiles is exactly what one would expect according to the ARRHENIUS temperature law, and the argument that thermophiles show a restricted respiration for that temperature (45°) is probably not justified.—C. A. SHULL.

---

[11] NOACK, KURT, Die Betriebstoffwechsel der thermophilen Pilze. Jahrb. Wiss. Bot. **95**:413–466. 1920.

[12] MATISSE, GEORGES, La loi d'Arrhenius contre la règle du coefficient de température. Archiv. Int. Physiol. **16**:461–466. 1921.

Salts and permeability to acids.—BRENNER[13] finds by the use of a deplasmolitic method on red cabbage that neutral salts modify the toxicity of acids. The following table shows the killing concentration of HCl, four hours exposure, following plasmolysis by the various salts:

Plasmolytic agent	Critical conc. of HCl in mols.	H ion conc. of critical sol.
NaCl 2.2 per cent	1/1000	8.91×10^{-4}
KNO_3 3.75 per cent	1/800	1.29×10^{-3}
KCl 2.8 per cent	1/600	1.38×10^{-3}
K_2SO_4 5.0 per cent	1/400	4.68×10^{-4}
$Mg(NO_3)_2$+6 aq. 8.8 per cent	1/1000	1.09×10^{-3}
$MgCl_2$+6 aq. 7.0 per cent	1/400	3.16×10^{-3}
$MgSO_4$+7 aq. 16.1 per cent	1/250	1.12×10^{-3}
$Ca(NO_3)_2$+4 aq. 6.5 per cent	1/500	1.95×10^{-3}
$CaCl_2$+6 aq. 6.2 per cent	1/250	5.50×10^{-3}
Dextrose	1/700	8.90×10^{-4}
Saccharose	1/700	8.71×10^{-4}

The author emphasizes the fact that salts antagonize the toxic action of strong mineral acid, H ion, just as they have long been known to do with other salts. The antagonistic action of salts toward H ions is due to the joint action of cations and anions of the salts. By change of color in the anthocyanin of the cells used, the author determined that acids enter uninjured cells very slowly, and that the effect of salts in reducing this entrance corresponds to their antitoxic effects. In cells that are killed by acids, NaCl, KCl, and KNO_3 favor the exosmose of anthocyanin, and salts of earth alkali delay it very much. Of the plasmolytic agents Mg salts proved very toxic. In $Mg(NO_3)_2$ no cells were alive after twenty-four hours, and in $MgCl_2$ and $MgSO_4$ very few. The salts of alkalis were only slightly less toxic, except for KCl, which showed many cells alive after two days. In $CaCl_2$ the cells would remain alive and plasmolyzed for a much longer time, some of them for twenty-one days. The author emphasizes the toxic action of pure salts and the balanced or non-toxic nature of mixtures of salts.—WM. CROCKER.

Soil moisture.—A new classification of soil moisture, based upon its behavior in freezing, appears to be founded upon scientific principles and to give a deeper insight into the actual condition of such water, its movement, and its relationship to plants. BOUYOUCOS[14] has found that a portion of the soil moisture freezes readily near 0°C., another portion only when a temperature of −4°C. is reached, and a third portion does not freeze at all. The first

---

[13] BRENNER, W., Über die Wirkung von Neutralsalzen auf die Säureresisteng, Permeabilität und Lebensdauer der Protoplasten. Ber. Deutsch Bot. Gesells. **38**: 277–285. 1921.

[14] BOUYOUCOS, G., A new classification of soil moisture. Soil Science **11**:33–47. 1921.

portion is called "free" water, to distinguish it from the remaining "unfree" water. The portion of the latter capable of being frozen is regarded as capillary-adsorbed, while that which does not freeze at all is the combined water or the water of solid solution and of hydration.

The physiological and ecological significance of such a classification is indicated by showing that a close relationship exists between the unfree water and the wilting coefficient of BRIGGS and SHANTZ, and between the combined water and the hygroscopic coefficient. The new classification, together with the relationship of the different classes of soil moisture to plants, may be concisely expressed as follows, gravitational water being the same as in older systems of classification:

1. Gravitational ..... superavailable
2. Free ..... very available
3. Unfree
   - Capillary-adsorbed ..... slightly available
   - Combined
     - water of solid solution
     - water of hydration

     ..... unavailable

The method of measuring the relative amounts of these various forms of water in the soil is known as the dilatometer method, and is relatively simple, being based upon the expansion of water upon freezing. This method would also seem to offer a convenient, rapid, and accurate method of determining the wilting coefficient.—GEO. D. FULLER.

Nutrients for Rhizopus.—Miss DUNN[15] has studied the effect of various concentrations and proportions of nutrients upon BLAKESLEE'S two races (male and female) of *Rhizopus nigricans*. The salts KH_2PO_4, NH_4NO_3, $MgSO_4$, and $FePO_4$ were used in various proportions, and total concentration with glucose or glycerine as the carbon source. Apparently calcium is not needed by this plant, and it makes no use of nitrate as a nitrogen source, but uses the NH_4 ion. Under the conditions of this investigation "the activity of the organisms appears clearly to be controlled by a combination of (*a*) salt proportions (or perhaps ion proportions), (*b*) total salt concentration, and (*c*) dextrose concentration. When these three conditions are poorly balanced for the growth of these races, the solution may sometimes be greatly improved by altering just one of the conditions, but it is frequently necessary to alter two conditions simultaneously to obtain good physiological balance. The solution representing the best combination has the following characteristics: (*a*) Molecular salt proportions: KH_2PO_4 6.0 : NH_4NO_3 1.0 : $MgSO_4$ 1.1 : $FePO_4$ a mere trace. (*b*) Total salt concentration, equivalent to a calculated osmotic value of 14.5 atmospheres. (*c*) Dextrose concentration, 1.0 gram-mol. per liter." There was no consistent difference in dry weight production between the male and female races when grown on dextrose, but when grown with glycerine as the

[15] DUNN, GRACE A., A comparative study of the two races of *Rhizopus nigricans*. Physiol. Researches 2:301–339. 1921.

carbon source, the male uniformly gave the higher yield. In all dextrose cultures where the yields were medium or high, the male showed much more sporangium production than the female, and the female was more vigorous in mycelial production. The male seems to have a somewhat higher maximum temperature than the female. The optimum for the female also seems to be somewhat lower.—WM. CROCKER.

Amylase of Rhizopus.—HARTER[16] has made a study of the amylase of *Rhizopus tritici*, particularly the effect of various factors on its secretion and action. The best growth of the fungus, and also secretion of amylase, as indicated by the amount of hydrolysis of starch, was obtained with CZAPECK'S nutrient solution, when ammonium nitrate was the source of the nitrogen, and starch the source of the carbon. Poorer results were obtained when sodium nitrate was used as the source of the nitrogen, and either cane sugar or glucose as the carbon source. Sweet potato bouillon, however, which contained both glucose and starch, gave the best results of all. The nutrient solution best for the growth of the fungus was also best for the secretion of the enzyme. The best temperature for the action of the enzyme was 45° C., and its action was practically nil at 60°. The secretion of the enzyme, as measured by the hydrolytic power of a unit weight of the enzyme powder, was much less when the fungus was grown at 40° C., the maximum temperature for its growth, than when it was grown at 9° C., which represents about the minimum temperature for its growth. While most of the experiments, the data from which are recorded in this paper, have been tried out by other workers, using other species of fungi, this seems to be the first time that *Rhizopus tritici* has been used for such an investigation.—S. V. EATON.

Plants of Mississippi.—The greater part of a useful little volume by LOWE[17] is occupied by an annotated list of the vascular plants of Mississippi, compiled from the ALLISON and TRACEY herbaria and from the field work and collections of the author and his assistant, THOMAS L. BAILEY. A feature of the work which should appeal to the general reader is an introduction comprising an elementary discussion of the problems of plant ecology expressed in non-technical language. Botanists will be more interested in the division of the state into the ten following topographic and floristic regions: (1) Tennessee River hills, (2) Northwestern prairie belt, (3) Pontotoc ridges, (4) Flatwoods, (5) North central plateau, (6) Jackson prairie belt, (7) Loess or bluff hills, (8) Yazoo-Mississippi delta, (9) Long leaf pine belt, (10) Coastal pine meadows. The topography, soil, and vegetation of each of these subdivisions are briefly described and lists of characteristic species given. The usefulness of the volume would be decidedly increased by supplementing the table of contents with an adequate index.—GEO. D. FULLER.

[16] HARTER, L. L., Amylase of *Rhizopus tritici*, with a consideration of its secretion and action. Jour. Agric. Res. **20**:761–786. 1921.

[17] LOWE, E. N., Plants of Mississippi. Miss. Geol. Surv. Bull. 17. pp. 292. 1921.

Bahaman endemics.—The Bahama Islands have been found to possess an endemic flora of some 185 species, or rather more than fourteen per cent of the entire plant population. There is but a single endemic genus, however, *Neobracea*, belonging to the Apocynaceae. A careful analysis of the situation by TAYLOR[18] shows that the distribution of these forms does not coincide with the "age and area" hypothesis of WILLIS, nor do the distribution and growth forms of the endemic differ materially from those of the non-endemic species. Moreover, the comparatively youthful land surface seems to preclude the idea of any considerable number of relic species. TAYLOR is thus forced to the conclusion that the endemics are due largely to the rather direct influence of the somewhat peculiar set of external factors that include sterile and often saline soil, deficient rainfall, strong trade winds varied by violent hurricanes, and possibly certain other factors.—GEO. D. FULLER.

Action of lichens on glass.—Doubt has sometimes been cast on the capacity of lichens to disintegrate the harder rocks. In this connection Miss MELLOR[19] reports the occurrence of twenty-two forms growing on the glass of church windows in France. The plants not only etched the glass, but produced pits up to 0.5 cm. in diameter and as much as 1.6 mm. deep, in the process chipping off fine fragments which became imbedded in their tissues. The action is explained as a purely mechanical result of the solution in rain water of the CO_2 excreted as a result of the respiration of the lichens, and is very slow, but the conclusion is drawn that plants able to attack glass in this way would have a similar disorganizing effect on rocks. In fact, one of the species found growing on glass was also found growing on slate, in which situation fine chips of slate were incorporated in the thallus.—G. W. MARTIN.

Zoölogisch-Botanische Gesellschaft.—This society is one of the oldest and most famous of the natural science societies of Europe. In order to continue its existence, it is compelled to sell some of its herbarium collections. In view of this need we publish the following notice received from Vienna:

"For sale—a valuable collection of mosses containing more than 1000 European species, including about 12,000 fine specimens collected by famous bryologists, as SCHIMPER, WILSON, DE NOTARIS, LINDBERG, JURATZKA, MOLENDO, LORENTZ, BREIDLER, many of them being types. The collection also includes several hundred members of old rare exsiccatae, as, for example, RABENHORST, Bryotheca Europaea. Offers should be sent to the Zoölogisch-Botanische Gesellschaft, III/3, Mechelgasse 2, Vienna, Austria."

---

[18] TAYLOR, NORMAN, Endemism in the Bahama flora. Ann. Botany **35**: 523–532. 1921.

[19] MELLOR, ETHEL, Les lichens vitricoles et leur action mécanique sur less vitraux d'église. Comptes Rend. Acad. Sci. **173**: 1106–1108. 1921.

Embryology.—SOUÈGES, in continuation of his work on the embryology of Angiosperms, has published several recent contributions. *Rheum Emodi*[20] is presented as a further representative of the Polygonaceae. *Chenopodium Bonus-Henricus*[21] is the subject of an unusually full treatment. In the presentation of the embryogeny of the Labiatae, *Glechoma hederacea* and *Lamium purpureum* form the subject of one paper,[22] and in the second paper[23] *Mentha viridis* is used. The Scrophulariaceae are represented by *Veronica arvensis*.[24] The presentation of the embryogeny of *Urtica pilulifera*[25] is completed with two papers.—J. M. C.

Overgrowth of stumps.—The overgrowth of the upper ends of stumps of *Pseudotsuga* and of *Abies grandis* has been observed by PEMBERTON[26] in British Columbia. Careful excavation showed that in every case root grafting with living trees existed, the connected roots providing an avenue for food conduction to the stump, and this he believes to be the true explanation of the interesting phenomenon.—GEO. D. FULLER.

Addisonia.—The fourth number completing Vol. 5 of this publication appeared in December 1920, and the first number of Vol. 6 appeared in March 1921. The fine colored illustrations and popular descriptions form a collection that should be in every botanical library.—J. M. C.

Potassium and growth of plants.—SMITH and BUTLER[27] have published a report of rather extensive work on the effect of potassium on the growth of plants. It confirms all the old findings and gives quantitative data on several new plants.—WM. CROCKER.

---

[20] SOUÈGES, R., Recherches sur l'embryogénie des Polygonacées (suite). Bull. Soc. Bot. France **67**: 75–85. 1920.

[21] ———, Développement de l'embryon chez le *Chenopodium Bonus-Henricus* L. *Ibid.* **67**: 233–257. *figs. 40.* 1920.

[22] ———, Embryogénie des Labiées. Développement de l'embryon chez le *Glechoma hederacea* L. et *Lamium purpureum* L. Compt. Rend 1921: 48–50.

[23] ———, Développement de l'embryon chez le *Mentha viridis* L. *Ibid.* 1921: 1057, 1058.

[24] ———, Embryogénie des Scrofulariacées. Développement de l'embryon chez le *Veronica arvensis* L. *Ibid.* 1921: 703–705.

[25] ———, Développement de l'embryon chez l'*Urtica pilulifera* L. Bull. Soc. Bot. France **68**: 172–188; 280–294. *figs. 57.* 1921.

[26] PEMBERTON, C. C., Overgrowth of stumps of conifers. Can. Field Nat. **35**: 81–87. *figs. 4.* 1921.

[27] SMITH, T. O., and BUTLER, O., Relation of potassium to growth in plants. Ann. Botany **35**: 189–225. 1921.

The University of Chicago

Nature-Study Series

By ELLIOT R. DOWNING

Associate Professor of Natural Science in the School of Education of the University of Chicago

Already Published

A Source Book of Biological Nature-Study

Cloth, $3.00; postpaid $3.20

The material is organized with special reference to the training of the teacher and the presentation of the subject to the pupil. In all the varied uses to which it may be put the 338 sketches and photographs add greatly to its effectiveness and charm.

"One fact strikes us with overwhelming force—namely, how immense an array of valuable and instructive matter can be presented to the student without a trace of dullness or pedantry . . . . the volume is literally a moving picture of natural history and a wide open door to vaster and more comprehensive fields of research."—*Chicago Daily News* for June 4, 1919.

A Field and Laboratory Guide in Biological Nature-Study

Paper covers, loose leaf, $1.50; postpaid $1.60

Intended for the use of students who are preparing to teach, and of teachers themselves. Deals with those groups of plants and animals that are of most interest to pupils.

"It gives specific suggestions and directions for field work. There are three keys: to autumn weeds, to trees, and to birds. There is much data as to methods given throughout the book, the outcome of years of work, which, brought together as it is under one cover, is invaluable to the inexperienced teacher."—*School Science and Mathematics* for May, 1919.

A Field and Laboratory Guide in Physical Nature-Study

Paper cover, loose leaf, $1.50; postpaid $1.60

Designed to acquaint the student with the rich field of physical science so often neglected in nature-study because of overemphasis on living things. The material here presented has been used by the author for many years in his teacher-training classes.

"A wise teacher will find many ways in which to use this manual, and many a boy or girl will find its pages suggestive and delightful."—*Education*, May, 1920.

"It should be in the hands of every teacher in the land who has to do with seventh and eighth grades or with high-school physics."—*Nature-Study Review* for April, 1920.

A Naturalist in the Great Lakes Region

Flexible binding, pocket size, $3.50; postpaid $3.70

This book gives an interesting description of the flora and fauna of the Great Lakes region and traces the development of the geological and physiographic factors which have affected the distribution of the plants and animals.

Other Volumes Are in Preparation

Address Correspondence to

THE UNIVERSITY OF CHICAGO PRESS

5822 Ellis Avenue **Chicago, Illinois**

It makes all Writing Swift and Easy

Remington Portable

A little brother of the big Remington. Small but strong. Light but efficient. Compact but complete.

Has every feature common to the big machines—including *automatic ribbon reverse* and *standard keyboard*.

Price, with case, $60

Remington Typewriter Company
(Incorporated)
374 Broadway
New York

Branches Everywhere

The Teaching of General Science

By W. L. EIKENBERRY
Associate Professor of General Science
University of Kansas

In this book the author discusses the necessity of the creation of a technique of presentation that will properly diffuse scientific knowledge. Detailed consideration is given to experiments in the instruction of the public in which the character of the general science movement, as well as its connection with the past history of science teaching, its relation to the established sciences, and its place in the new science of education, is depicted.

This volume is not a manual of class-room methods. It contains the subject-matter of the general science course, together with the objectives and principles of organization of general science. The author has provided a brief bibliography of periodical literature of general science.

Cloth, $2.00, postpaid $2.15

The University of Chicago Press
Chicago - - - - Illinois

Advantageous Features

SPENCER MICROSCOPES

EVERY SPENCER MICROSCOPE MADE DURING THE PAST 15 OR MORE YEARS HAS POSSESSED THE FOLLOWING ADVANTAGEOUS FEATURES:

I. Objective Lenses mounted directly into the metal mount, avoiding the use of Canada balsam to hold them.

II. Fine adjustment so constructed as to avoid breakage of cover glass when focused down upon it.

THESE ARE BUT TWO OF THE MANY ADVANTAGES INCORPORATED INTO SPENCER MICROSCOPES, WHICH EVER HAVE THE VERY LATEST AND BEST IMPROVEMENTS.

SPENCER BUFFALO U.S.A.

SPENCER LENS COMPANY

Manufacturers
Microscopes, Microtomes, Delineascopes, Optical Glass, Telescope Discs and Lenses, Optical Measuring Instruments, Etc.
BUFFALO, N.Y.

SPENCER BUFFALO U.S.A.